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L5 ANSWER 1 OF 6 CAPLUS COPYRIGHT 2001 ACS
2001:380427 Document No. 135:496 Immunomodulatory compositions containing an
immunostimulatory sequence linked to antigen and methods of use thereof.
Tuck, Stephen; Van Nest, Gary (Dynavax Technologies Corporation, USA). PCT Int. Appl. WO 2001035991 A2 20010525, 97 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2000-US31385 20001115. PRIORITY: US 1999-PV165467 19991115; US 2000-713136 20001114.

AB The invention provides classes of immunomodulatory compns. which comprise an av. of one or more immunostimulatory sequence (ISS) contg. polynucleotide conjugated, or attached, to antigen. The extent of conjugation affects immunomodulatory properties, such as extent of antigen-specific antibody formation, including Th1-assocd. antibody formation, and thus these various conjugate classes are useful for modulating the type and extent of immune response. The invention also includes methods of modulating an immune response using these compns.

L5 ANSWER 2 OF 6 MEDLINE DUPLICATE 1
2001450277 Document Number: 21387316. PubMed ID: 11496233.
Immunostimulatory sequence DNA linked to the Amb a 1 **allergen** promotes T(H)1 cytokine expression while downregulating T(H)2 cytokine expression in PBMCs from human patients with ragweed allergy. Marshall J D; Abtahi S; Eiden J J; **Tuck S**; Milley R; Haycock F; Reid M J; Kagey-Sobotka A; Creticos P S; Lichtenstein L M; Van Nest G. (Dynavax Technologies Corporation, Berkeley, California 94710, USA.) JOURNAL OF ALLERGY AND CLINICAL IMMUNOLOGY, (2001 Aug) 108 (2) 191-7. Journal code: H53; 1275002. ISSN: 0091-6749. Pub. country: United States. Language: English.

AB BACKGROUND: Recent studies have demonstrated that bacterially derived immunostimulatory sequences (ISSs) of DNA can activate the mammalian innate immune system and promote the development of T(H)1 cells. Promotion of T(H)1 immunity by means of immunotherapy in allergic patients has led to the alleviation of symptoms that result from **allergen**-specific T(H)2 responses. OBJECTIVE: Our purpose was to investigate whether the T(H)1-enhancing properties of ISSs could be used to alter the T(H)2-dominated immune response of allergic PBMCs in vitro. METHODS: Ragweed protein-linked ISS (PLI) was generated from a specific, highly active 22-base ISS and Amb a 1, the immunodominant **allergen** in ragweed pollen, to combine the T(H)1-enhancing properties of ISSs with **allergen** selectivity, and its activity was investigated in PBMC cultures from subjects with ragweed allergy. RESULTS: PLI was markedly successful at reversing the dominant **allergen**-induced T(H)2 profile while greatly enhancing IFN-gamma production. Delivering ISSs in a linked form proved to be much more effective at modulating the resulting cytokine profile than delivering free ISSs in a mixture with unlinked Amb a 1. PLI also demonstrated cytokine-modulating properties, even when used to stimulate cells that had already been primed for 6 days with Amb a 1. The antigen specificity of the action of PLI was confirmed by the observations that PLI enhances Amb a 1--specific T-cell proliferation. CONCLUSION: These data indicate that delivery of ISSs within an antigen-specific context exhibits potent cytokine-modulating activity and, combined with its reduced allergenicity, makes this molecule a strong candidate for use in improved immunotherapy applications.

L5 ANSWER 3 OF 6 SCISEARCH COPYRIGHT 2001 ISI (R)
2000:191596 The Genuine Article (R) Number: 287WR. Conjugation of immunostimulatory DNA (ISS) to the major short ragweed **allergen**, Amb a 1, enhances immunogenicity and reduces allergenicity.. VanNest G (Reprint); Eiden J J; **Tuck S F**; KageySobarka A; Creticos P S; Lichtenstein L M; Spiegelberg H L; Raz E. JOHNS HOPKINS UNIV, BALTIMORE, MD 21218; UNIV CALIF SAN DIEGO, LA JOLLA, CA 92093. JOURNAL OF ALLERGY AND CLINICAL IMMUNOLOGY (JAN 2000) Vol. 105, No. 1, Part 2, Supp. [S], pp. 216-216. Publisher: MOSBY-YEAR BOOK INC. 11830 WESTLINE INDUSTRIAL DR, ST LOUIS, MO 63146-3318. ISSN: 0091-6749. Pub. country: USA. Language: English.

L5 ANSWER 4 OF 6 MEDLINE DUPLICATE 2
2000387434 Document Number: 20347064. PubMed ID: 10887315. Conjugation of immunostimulatory DNA to the short ragweed **allergen** amb a 1 enhances its immunogenicity and reduces its allergenicity. Tighe H; Takabayashi K; Schwartz D; Van Nest G; **Tuck S**; Eiden J J; Kagey-Sobotka A; Creticos P S; Lichtenstein L M; Spiegelberg H L; Raz E. (Department of Medicine and The Sam and Rose Stein Institute for Research on Aging, and the Department of Pediatrics, University of California San Diego School of Medicine, La Jolla, CA, USA.) JOURNAL OF ALLERGY AND CLINICAL IMMUNOLOGY, (2000 Jul) 106 (1 Pt 1) 124-34. Journal code: H53;

1275002. ISSN: 0091-6749. Pub. country: United States. Language: English.
AB BACKGROUND: **Allergen** immunotherapy is inconvenient and associated with the risk of anaphylaxis. Efforts to improve the safety of immunotherapy by means of chemical modification of **allergens** have not been successful because it greatly reduced their antigenicity. Recently, immunostimulatory DNA sequences (ISS or CpG motifs) have been shown to act as strong T(H)1 response-inducing adjuvants. OBJECTIVE: We sought to determine whether conjugation of ISS to the major short ragweed **allergen** Amb a 1 results in enhanced immunotherapeutic potential in mice and decreased allergenicity in human subjects. METHODS: A 22-mer ISS oligodeoxynucleotide (ISS-ODN) was coupled to Amb a 1 and used for immunization of mice, rabbits, and monkeys. RESULTS: In mice the Amb a 1-ISS conjugate induced a T(H)1 response (IFN-gamma secretion), whereas Amb a 1 induced a T(H)2 response (IL-5 secretion). The T(H)1 response was not observed with an Amb a 1-non-ISS conjugate. Coinjection of Amb a 1 with ISS-ODN was much less effective in inducing a T(H)1 response. In mice primed for a T(H)2 response, injection with Amb a 1-ISS conjugate induced a de novo T(H)1 response and suppressed IgE antibody formation after challenge with Amb a 1. Amb a 1-ISS conjugate induced high-titer anti-Amb a 1 IgG antibodies in rabbits and cynomolgus monkeys, whereas Amb a 1 alone or Amb a 1 coinjected with ISS-ODN did not induce a detectable response. Amb a 1-ISS conjugate was less allergenic than Amb a 1 alone, as shown by a 30-fold lower histamine release from human basophils of patients with ragweed allergy, whereas mixing ISS-ODN with Amb a 1 did not reduce histamine release. CONCLUSION: Amb a 1-ISS conjugate has an enhanced T(H)1-biased immunogenicity and reduced allergenicity. It may offer a more effective and safer approach for **allergen** immunotherapy than currently available methods.

L5 ANSWER 5 OF 6 BIOSIS COPYRIGHT 2001 BIOSIS
2000:137708 Document No.: PREV200000137708. Immunostimulatory oligonucleotides conjugated to Amb a 1: Safety, skin test reactivity, and basophil histamine release. Creticos, P. S. (1); Eiden, J. J.; Balcer, S. L. (1); Van Nest, G.; Kagey-Sobotka, A. (1); **Tuck, S. F.**; Norman, P. S. (1); Lichtenstein, L. M. (1). (1) Johns Hopkins University, Baltimore, MD USA. Journal of Allergy and Clinical Immunology., (Jan., 2000) Vol. 105, No. 1 part 2, pp. S70. Meeting Info.: 56th Annual Meeting of the American Academy of Allergy, Asthma and Immunology. San Diego, California, USA March 03-08, 2000 American Academy of Allergy, Asthma and Immunology. ISSN: 0091-6749. Language: English. Summary Language: English.

L5 ANSWER 6 OF 6 BIOSIS COPYRIGHT 2001 BIOSIS
2000:140208 Document No.: PREV200000140208. Conjugation of immunostimulatory DNA (ISS) to the major short ragweed **allergen**, Amb a 1, enhances immunogenicity and reduces allergenicity. Van Nest, G.; Eiden, J. J.; **Tuck, S. F.**; Kagey-Sobatka, A.; Creticos, P. S.; Lichtenstein, L. M.; Spiegelberg, H. L.; Raz, E.. Journal of Allergy and Clinical Immunology., (Jan., 2000) Vol. 105, No. 1 part 2, pp. S70. Meeting Info.: 56th Annual Meeting of the American Academy of Allergy, Asthma and Immunology. San Diego, California, USA March 03-08, 2000 American Academy of Allergy, Asthma and Immunology. ISSN: 0091-6749. Language: English. Summary Language: English.

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L6 9312 "ISS"

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L7 0 L5 AND IMMUNOSTIMULATORY SEUQENCE

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L9 ANSWER 1 OF 8 CAPLUS COPYRIGHT 2001 ACS
2001:693110 Methods of preventing and treating respiratory viral infection using immunomodulatory **polynucleotide** sequences. Van Nest, Gary (Dynavax Technologies Corporation, USA). PCT Int. Appl. WO 2001068116 A2 20010920, 40 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2001-US7839 20010312. PRIORITY: US 2000-PV188583 20000310; US 2001-802686 20010309.
AB The invention provides methods of preventing and/or treating infection by a respiratory virus such as respiratory syncytial virus (RSV), particularly reducing infection and/or one or more symptoms of respiratory virus infection. A **polynucleotide** comprising an immunostimulatory sequence (an "**ISS**") is administered to an individual which is at risk of being exposed to a respiratory virus, has been exposed to a respiratory virus or is infected with a respiratory virus. The **ISS** is administered without any antigens of the respiratory virus. Administration of the **ISS** results in reduced incidence and/or severity of one or more symptoms of respiratory virus infection.

L9 ANSWER 2 OF 8 CAPLUS COPYRIGHT 2001 ACS
2001:693098 Methods of ameliorating symptoms of herpes infection using immunomodulatory **polynucleotide** sequences. Van Nest, Gary (Dynavax Technologies Corporation, USA). PCT Int. Appl. WO 2001068103 A2 20010920, 49 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2001-US7841 20010312. PRIORITY: US 2000-PV188556 20000310; US 2001-802518 20010309.
AB The invention provides new methods of preventing and/or treating herpes virus infections, particularly reducing infection, one or more symptoms and recurrence of one or more symptoms of herpes simplex virus infection. A **polynucleotide** comprising an immunostimulatory sequence (an "**ISS**") is administered to an individual which is at risk of being exposed to .alpha.-herpesvirinae, has been exposed to .alpha.-herpesvirinae or is infected with .alpha.-herpesvirinae. The **ISS** is administered without any .alpha.-herpesvirinae antigens. Administration of the **ISS** results in reduced incidence,

recurrence, and severity of one or more symptoms of .alpha.-herpesvirinae infection.

L9 ANSWER 3 OF 8 CAPLUS COPYRIGHT 2001 ACS
2001:693074 Methods of preventing and treating viral infections and using immunomodulatory **polynucleotide** sequences. Van Nest, Gary (Dynavax Technologies Corporation, USA). PCT Int. Appl. WO 2001068077 A2 20010920, 65 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SK, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2001-US7840 20010312. PRIORITY: US 2000-PV188302 20000310; US 2001-802685 20010309.
AB The invention provides methods of suppression, prevention, and/or treatment of infection by viruses. A **polynucleotide** comprising an immunostimulatory sequence (an "**ISS**") is administered to an individual who is at risk of being exposed to, has been exposed to or is infected with a virus. The **ISS**-contg. **polynucleotide** is administered without any antigens of the virus. Administration of the **ISS**-contg. **polynucleotide** results in reduced incidence and/or severity of one or more symptoms of virus infection.

L9 ANSWER 4 OF 8 CAPLUS COPYRIGHT 2001 ACS
2001:380427 Document No. 135:496 Immunomodulatory compositions containing an
immunostimulatory sequence linked to antigen and methods of use thereof. Tuck, Stephen; Van Nest, Gary (Dynavax Technologies Corporation, USA). PCT Int. Appl. WO 2001035991 A2 20010525, 97 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2000-US31385 20001115. PRIORITY: US 1999-PV165467 19991115; US 2000-713136 20001114.
AB The invention provides classes of immunomodulatory compns. which comprise an av. of one or more immunostimulatory sequence (**ISS**) contg. **polynucleotide** conjugated, or attached, to antigen. The extent of conjugation affects immunomodulatory properties, such as extent of antigen-specific antibody formation, including Th1-assocd. antibody formation, and thus these various conjugate classes are useful for modulating the type and extent of immune response. The invention also includes methods of modulating an immune response using these compns.

L9 ANSWER 5 OF 8 CAPLUS COPYRIGHT 2001 ACS
2000:209943 Document No. 132:246356 Methods and compositions using an IgE inhibitor and an antigen and/or immunostimulatory **polynucleotide** for treating IgE-associated disorders. Dina, Dino (Dynavax Technologies Corporation, USA). PCT Int. Appl. WO 2000016804 A1 20000330, 42 pp. DESIGNATED STATES: W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH,
CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1999-US21686 19990917. PRIORITY: US

1998-100838 19980918; US 1999-136600 19990528; US 1999-397198 19990916.

AB The invention provides methods of treating IgE-assocd. disorders and compns. for use therein. The methods are particularly useful in treatment of allergies and allergy-related disorders. The methods generally comprise administering an IgE inhibitor (such as anti-IgE antibody) and an antigen and/or immunostimulatory **polynucleotide** sequence (**ISS**). These combination methods offer significant advantages, such as allowing more aggressive therapy while reducing unwanted side effects, such as anaphylaxis.

L9 ANSWER 6 OF 8 CAPLUS COPYRIGHT 2001 ACS

1998:251073 Document No. 129:40137 Immunostimulatory **polynucleotide** /immunomodulatory molecule conjugates. Carson, Dennis A.; Raz, Eyal; Roman, Mark (Regents of the University of California, USA; Carson, Dennis A.; Raz, Eyal; Roman, Mark). PCT Int. Appl. WO 9816247 A1 19980423, 69 pp. DESIGNATED STATES: W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1997-US19004 19971009. PRIORITY: US 1996-28118 19961011.

AB Immunostimulatory **polynucleotide**-immunomodulatory mol. conjugate compns. are disclosed for enhancing Th1 cellular immune response during humoral immune response induction by antigen. These compns. include a **polynucleotide** that is linked to an immunomodulatory mol., which mol. comprises an antigen and may further comprise immunomodulators such as cytokines and adjuvants. The **polynucleotide** portion of the conjugate includes at least one immunostimulatory oligonucleotide nucleotide sequence (**ISS**). Methods of modulating an immune response upon administration of the **polynucleotide** -immunomodulatory conjugate prepn. to a vertebrate host are also disclosed. The method is useful for tumor therapy, for treating allergic disorders and other conditions.

L9 ANSWER 7 OF 8 BIOSIS COPYRIGHT 2001 BIOSIS

1992:138578 Document No.: BA93:72803. METHOD OF ARTIFICIAL DNA SPLICING BY DIRECTED LIGATION SDL. LEBEDENKO E N; BIRIKH K R; PLUTALOV O V; BERLIN YU A. M.M. SHEMYAKIN INST. BIOORGANIC CHEM., ACADEMY SCI. USSR, MOSCOW 117871.. NUCLEIC ACIDS RES, (1991) 19 (24), 6757-6762. CODEN: NARHAD. ISSN: 0305-1048. Language: English.

AB An approach to directed genetic recombination in vitro has been devised, which allows for joining together, in a predetermined way, a series of DNA segments to give a precisely spliced **polynucleotide** sequence (DNA splicing by directed ligated, SDL). The approach makes use of amplification, by means of several polymerase chain reactions (PCR), of a chosen set of DNA segments. Primers for the amplifications contain recognition sites of the class **ISS** restriction endonucleases, which transform blunt ends of the amplification products into protruding ends of unique primary structures, the ends to be used for joining segments together being mutually complementary. Ligation of the mixture of the segments so synthesized gives the desired sequence in an unambiguous way. The suggested approach has been exemplified by the synthesis of a totally processed (intronless) gene encoding human mature interleukin-1.alpha..

L9 ANSWER 8 OF 8 CAPLUS COPYRIGHT 2001 ACS

1982:99806 Document No. 96:99806 Hunting of strange electronic states and structures in solids and biopolymers. Biczo, G.; Lukovits, I. (Cent. Res.

Inst. Chem., Hung. Acad. Sci., Budapest, H-1525, Hung.). Int. J. Quantum Chem., Quantum Biol. Symp., 8, 347-57 (English) 1981. CODEN: IJQBDZ. ISSN: 0360-8832.

AB The existence problem of the intermediate states (IS) possibly appearing in bounded crystals with perfect bulk structure and of certain zig-zag states (ZZS) in almost periodic (even disordered) biopolymers is discussed. An example is given of how to enhance the appearance probability of **ISs**. Within a narrow crit. interval, the width of which is .apprx.1/8 of the av. sepn. of the bulk states in the k space of the finite linear chains, any state becomes an AIS (almost IS), which is very similar to the IS and approaches it continuously. Estg. the effect of in-plane and interplane vibrations of stacked arom. planar compds. on the delocalized .pi.-electron structures, it is concluded that the valence modes have a strong effect on the in-plane .pi.-electronic systems, but a very small effect on the interplane .pi.-electronic interactions. The longitudinal acoustical modes of finite **polynucleotide** DNA models and their root mean square amplitudes are estd. by the method of W. L. Peticolas (1979). According to the results obtained, DNA-like and layered graphitelike systems seem to be the probable host systems of **ISs**, if they exist at all. The appropriateness of the recursion method is discussed to search for unusual electronic states and structures. Such studies are the 1st unsteady but exciting steps toward the construction of the mol. computers.

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FILE 'MEDLINE, EMBASE, BIOSIS, SCISEARCH, CAPLUS' ENTERED AT 15:33:43 ON 09 OCT 2001

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L1          378 S TUCK S?/AU OR NEST G?/AU
L2            0 S L1 AND IMMUNOSTIMULATORY SEUQENCE
L3            0 S L1 AND RAGWEED POLLEN ALLERGEN
L4           14 S L1 AND ALLERGEN
L5            6 DUP REMOVE L4 (8 DUPLICATES REMOVED)
L6         9312 S "ISS"
L7            0 S L5 AND IMMUNOSTIMULATORY SEUQENCE
L8            8 S L6 AND POLYNUCLEOTIDE
L9            8 DUP REMOVE L8 (0 DUPLICATES REMOVED)
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=> s l6 and conjugate

L10 52 L6 AND CONJUGATE

=> s l10 and vaccine

L11 29 L10 AND VACCINE

=> s l11 and allergy

L12 1 L11 AND ALLERGY

=> d l12

L12 ANSWER 1 OF 1 MEDLINE
AN 2000387434 MEDLINE
DN 20347064 PubMed ID: 10887315
TI Conjugation of immunostimulatory DNA to the short ragweed allergen amb a 1

enhances its immunogenicity and reduces its allergenicity.
CM Comment in: J Allergy Clin Immunol. 2000 Jul;106(1 Pt 1):37-40
AU Tighe H; Takabayashi K; Schwartz D; Van Nest G; Tuck S; Eiden J J; Kagey-Sobotka A; Creticos P S; Lichtenstein L M; Spiegelberg H L; Raz E
CS Department of Medicine and The Sam and Rose Stein Institute for Research on Aging, and the Department of Pediatrics, University of California San Diego School of Medicine, La Jolla, CA, USA.
NC AI40682 (NIAID)
SO JOURNAL OF ALLERGY AND CLINICAL IMMUNOLOGY, (2000 Jul) 106 (1 Pt 1) 124-34.
Journal code: H53; 1275002. ISSN: 0091-6749.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Abridged Index Medicus Journals; Priority Journals
EM 200008
ED Entered STN: 20000818
Last Updated on STN: 20000928
Entered Medline: 20000809

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PROCESSING COMPLETED FOR L11
L13 10 DUP REMOVE L11 (19 DUPLICATES REMOVED)

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L13 ANSWER 1 OF 10 MEDLINE DUPLICATE 1
2001222550 Document Number: 21211991. PubMed ID: 11312000. The adjuvant effect of synthetic oligodeoxynucleotide containing CpG motif converts the anti-Haemophilus influenzae type b glycoconjugates into efficient anti-polysaccharide and anti-carrier polyvalent **vaccines**. von Hunolstein C; Mariotti S; Teloni R; Alfarone G; Romagnoli G; Orefici G; Nisini R. (Laboratorio di Batteriologia e Micologia Medica, Istituto Superiore di Sanita, Viale Regina Elena 299, 00161, Rome, Italy.) VACCINE, (2001 Apr 30) 19 (23-24) 3058-66. Journal code: X60; 8406899. ISSN: 0264-410X. Pub. country: England: United Kingdom. Language: English.
AB Synthetic oligodeoxynucleotides containing CpG immunostimulatory sequences (**ISS**) have been shown to act as potent adjuvants of type 1 immune responses when co-administered with protein or peptide **vaccines**. We have recently shown that **ISS** can increase the anti-polysaccharide (CHO) and anti-tetanus toxoid (TT) or anti-diphtheria (CRM) toxoid antibody levels if used as adjuvant of anti-Haemophilus influenzae type b (Hib) CHO **vaccine** conjugated with TT or CRM. The analysis of anti-TT and anti-CRM IgG subclasses showed a significant increase in IgG2a, IgG2b and/or IgG3 in the presence of **ISS**. Anti-TT and anti-CRM antibodies were shown to neutralize the activity of both the tetanus and diphtheria toxin in vivo or in vitro tests respectively. These data show that **ISS** have the potential to increase host antibody response against both the CHO and the protein component of a conjugated **vaccine**, and encourage the investigation to identify strategies of vaccination with schedules aimed at the valuation of protein carriers as protective immunogens.

L13 ANSWER 2 OF 10 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.DUPLICATE 2
2001270540 EMBASE Immunostimulatory DNA-based **vaccines** elicit multifaceted immune responses against HIV at systemic and mucosal sites.
Horner A.A.; Datta S.K.; Takabayashi K.; Belyakov I.M.; Hayashi T.; Cinman

N.; Nguyen M.-D.; Van Uden J.H.; Berzofsky J.A.; Richman D.D.; Raz E..
Dr.
E. Raz, Department of Medicine, Univ. of California at San Diego, 9500 Gilman Drive, San Diego, CA 92093-0663, United States. eraz@ucsd.edu.
Journal of Immunology 167/3 (1584-1591) 1 Aug 2001.
Refs: 62.
ISSN: 0022-1767. CODEN: JOIMA3. Pub. Country: United States. Language: English. Summary Language: English.
AB Immunostimulatory DNA sequences (**ISS**, also known as CpG motifs) are pathogen-associated molecular patterns that are potent stimulators of innate immunity. We tested the ability of **ISS** to act as an immunostimulatory pathogen-associated molecular pattern in a model HIV **vaccine** using gp120 envelope protein as the Ag. Mice immunized with gp120 and **ISS**, or a gp120:**ISS conjugate**, developed gp120-specific immune responses which included: 1) Ab production; 2) a Th1-biased cytokine response; 3) the secretion of .beta.-chemokines, which are known to inhibit the use of the CCR5 coreceptor by HIV; 4) CTL activity; 5) mucosal immune responses; and 6) CD8 T cell responses that were independent of CD4 T cell help. Based on these results, **ISS**-based immunization holds promise for the development of an effective preventive and therapeutic HIV **vaccine**.

L13 ANSWER 3 OF 10 MEDLINE DUPLICATE 3
2001408839 Document Number: 21187367. PubMed ID: 11292014. DNA-based immunotherapeutics for the treatment of allergic disease. Horner A A; Van Uden J H; Zubeldia J M; Broide D; Raz E. (Department of Medicine and The Sam and Rose Stein Institute for Research on Aging, University of California, San Diego, La Jolla 92093-0663, USA.) IMMUNOLOGICAL REVIEWS, (2001 Feb) 179 102-18. Ref: 72. Journal code: GG4; 7702118. ISSN: 0105-2896. Pub. country: Denmark. Language: English.
AB Allergic diseases are a growing health concern in industrialized countries. Despite a number of effective therapeutic options for the prevention and treatment of the pathophysiologic responses which characterize allergic diseases, the induction of true allergen desensitization remains an elusive therapeutic goal. Only immunotherapy (IT) has been shown to have any effect on the underlying hypersensitivities which mediate allergic reactions, and traditional protein-based allergen IT has a limited scope of efficacy However, a number of reagents collectively termed DNA-based immunotherapeutics have proven highly effective in both the prevention and reversal of Th2-mediated hypersensitivity states in mouse models of allergic disease. Four basic DNA-based immunotherapeutic modalities have been used for these studies. These include immunization with gene **vaccines**, allergen mixed with immunostimulatory oligodeoxynucleotide (**ISS**-ODN), and physical allergen-**ISS**-ODN **conjugates** (AIC), as well as immunomodulation with **ISS**-ODN alone. Results from many laboratories have generated guarded optimism that DNA-based immunotherapeutics may be effective for the reversal of allergic hypersensitivity states in humans, and several clinical trials have already been initiated. This review will focus on our present understanding of the biological activities of DNA-based immunotherapeutics and their application to the treatment of allergic diseases.

L13 ANSWER 4 OF 10 MEDLINE DUPLICATE 4
2000427428 Document Number: 20395232. PubMed ID: 10940883. Conjugation of protein to immunostimulatory DNA results in a rapid, long-lasting and potent induction of cell-mediated and humoral immunity. Tighe H; Takabayashi K; Schwartz D; Marsden R; Beck L; Corbeil J; Richman D D; Eiden J J Jr; Spiegelberg H L; Raz E. (Department of Medicine, The Sam and

Rose Stein Institute for Research of Aging, University of California, San Diego, La Jolla 92093-0663, USA.. htighe@ucsd.edu) . EUROPEAN JOURNAL OF IMMUNOLOGY, (2000 Jul) 30 (7) 1939-47. Journal code: EN5; 1273201. ISSN: 0014-2980. Pub. country: GERMANY: Germany, Federal Republic of. Language: English.

AB Immunostimulatory DNA sequences (**ISS**) are a potent Th1 adjuvant. We hypothesized that conjugation of **ISS** to protein antigens would strongly enhance their immunogenicity because both antigen and adjuvant (**ISS**) would be delivered to the same locale/antigen-presenting cell. To test this hypothesis, we conjugated a 22-mer immunostimulatory oligodeoxynucleotide (**ISS**-ODN) to two test antigens of differing intrinsic immunogenicity, namely Escherichia coli beta-galactosidase and the HIV-1 envelope glycoprotein gp120. We show that the antigen-**ISS conjugates** rapidly induce Th1 cells secreting high levels of IFN-gamma, strong CTL activity, and high titer IgG2a and HIV-neutralizing antibodies, exceeding gene and protein vaccination alone or immunization with mixtures of antigen and **ISS** -ODN. The data suggest that this procedure generates a novel and unique **vaccine** that rapidly triggers strong humoral and cell-mediated immunity.

L13 ANSWER 5 OF 10 MEDLINE DUPLICATE 5

2000264172 Document Number: 20264172. PubMed ID: 10802617. Immunostimulatory DNA-based **vaccines** induce cytotoxic lymphocyte activity by a T-helper cell-independent mechanism. Cho H J; Takabayashi K; Cheng P M; Nguyen M D; Corr M; Tuck S; Raz E. (Department of Internal Medicine and The Sam and Rose Stein Institute for Research on Aging, University of California San Diego, La Jolla, CA 92093-0663, USA.) NATURE BIOTECHNOLOGY, (2000 May) 18 (5) 509-14. Journal code: CQ3; 9604648. ISSN: 1087-0156. Pub. country: United States. Language: English.

AB Immunostimulatory DNA sequences (**ISS**) contain unmethylated CpG dinucleotides within a defined motif. Immunization with **ISS** -based **vaccines** has been shown to induce high antigen-specific cytotoxic lymphocyte (CTL) activity and a Th1-biased immune response. We have developed a novel **ISS**-based **vaccine** composed of ovalbumin (OVA) chemically conjugated to **ISS**-oligodeoxynucleotide (ODN). Protein-**ISS conjugate** (PIC) is more potent in priming CTL activity and Th1-biased immunity than other **ISS**-based **vaccines**. Cytotoxic lymphocyte activation by **ISS**-ODN-based **vaccines** is preserved in both CD4-/- and MHC class II-/- gene-deficient animals. Furthermore, PIC provides protection against a lethal burden of OVA-expressing tumor cells in a CD8+ cell-dependent manner. These results demonstrate that PIC acts through two unique mechanisms: T-helper-independent activation of CTL and facilitation of exogenous antigen presentation on MHC class I. This technology may have clinical applications in cancer therapy and in stimulating host defense in AIDS and chronic immunosuppression.

L13 ANSWER 6 OF 10 MEDLINE DUPLICATE 6

2000166981 Document Number: 20166981. PubMed ID: 10700464. Synthetic oligodeoxynucleotide containing CpG motif induces an anti-polysaccharide type 1-like immune response after immunization of mice with Haemophilus influenzae type b **conjugate vaccine**. von Hunolstein C; Teloni R; Mariotti S; Recchia S; Orefici G; Nisini R. (Laboratorio di Batteriologia e Micologia Medica, Istituto Superiore di Sanita, Viale Regina Elena 299, 00161 Roma, Italy.) INTERNATIONAL IMMUNOLOGY, (2000 Mar) 12 (3) 295-303. Journal code: AY5; 8916182. ISSN: 0953-8178. Pub. country: ENGLAND: United Kingdom. Language: English.

AB Synthetic oligodeoxynucleotides containing CpG motifs [immunostimulatory sequences (**ISS**)] have been described as potent adjuvants of type 1 immune responses when co-administered with protein or peptide

vaccines. To investigate their role in the immune response to polysaccharides (CHO), different preparations of anti-Haemophilus influenzae type b (Hib) **conjugate vaccine** were administered to mice. The unconjugated CHO did not induce the synthesis of specific antibodies even in the presence of **ISS**. On the other hand, anti-CHO-specific antibodies significantly increased in the presence of **ISS**, when tetanus (TT) or diphtheria [cross-reacting material (CRM)] toxoid-conjugated CHO were used to immunize mice. The adjuvant effect was also observed for the immune response against the carrier protein (TT and CRM). **ISS** insured an early and long-lasting specific IgG production. The effects of **ISS** on the anti-CHO immune response could be attributed to the amplification of the T help provided by the carrier. The analysis of anti-CHO IgG subclasses showed a significant increase of IgG2a and IgG3 in the presence of **ISS**. **ISS** caused a rapid release of IL-12 and IFN-gamma in sera from treated mice. This data provide a first evidence for the ability of **ISS** to induce an anti-CHO type 1-like immune response and demonstrate that **ISS** have the potential to increase host antibody response against both the CHO and the protein component of a conjugated **vaccine**.

L13 ANSWER 7 OF 10 MEDLINE
2000387434 Document Number: 20347064. PubMed ID: 10887315. Conjugation of immunostimulatory DNA to the short ragweed allergen amb a 1 enhances its immunogenicity and reduces its allergenicity. Tighe H; Takabayashi K; Schwartz D; Van Nest G; Tuck S; Eiden J J; Kagey-Sobotka A; Creticos P S; Lichtenstein L M; Spiegelberg H L; Raz E. (Department of Medicine and The Sam and Rose Stein Institute for Research on Aging, and the Department of Pediatrics, University of California San Diego School of Medicine, La Jolla, CA, USA.) JOURNAL OF ALLERGY AND CLINICAL IMMUNOLOGY, (2000 Jul) 106 (1 Pt 1) 124-34. Journal code: H53; 1275002. ISSN: 0091-6749. Pub. country: United States. Language: English.

AB BACKGROUND: Allergen immunotherapy is inconvenient and associated with the risk of anaphylaxis. Efforts to improve the safety of immunotherapy by means of chemical modification of allergens have not been successful because it greatly reduced their antigenicity. Recently, immunostimulatory DNA sequences (**ISS** or CpG motifs) have been shown to act as strong T(H)1 response-inducing adjuvants. OBJECTIVE: We sought to determine whether conjugation of **ISS** to the major short ragweed allergen Amb a 1 results in enhanced immunotherapeutic potential in mice and decreased allergenicity in human subjects. METHODS: A 22-mer **ISS** oligodeoxynucleotide (**ISS**-ODN) was coupled to Amb a 1 and used for immunization of mice, rabbits, and monkeys. RESULTS: In mice the Amb a 1-**ISS conjugate** induced a T(H)1 response (IFN-gamma secretion), whereas Amb a 1 induced a T(H)2 response (IL-5 secretion). The T(H)1 response was not observed with an Amb a 1-non-**ISS conjugate**. Coinjection of Amb a 1 with **ISS**-ODN was much less effective in inducing a T(H)1 response. In mice primed for a T(H)2 response, injection with Amb a 1-**ISS conjugate** induced a de novo T(H)1 response and suppressed IgE antibody formation after challenge with Amb a 1. Amb a 1-**ISS conjugate** induced high-titer anti-Amb a 1 IgG antibodies in rabbits and cynomolgus monkeys, whereas Amb a 1 alone or Amb a 1 coinjected with **ISS**-ODN did not induce a detectable response. Amb a 1-**ISS conjugate** was less allergenic than Amb a 1 alone, as shown by a 30-fold lower histamine release from human basophils of patients with ragweed allergy, whereas mixing **ISS**-ODN with Amb a 1 did not reduce histamine release. CONCLUSION: Amb a 1-**ISS**

conjugate has an enhanced T(H)1-biased immunogenicity and reduced allergenicity. It may offer a more effective and safer approach for allergen immunotherapy than currently available methods.

L13 ANSWER 8 OF 10 BIOSIS COPYRIGHT 2001 BIOSIS
1998:98255 Document No.: PREV199800098255. Correction of PREVIEWS 99554579. Randomised trial of Haemophilus influenzae type-b tetanus protein **conjugate vaccine** for prevention of pneumonia and meningitis in Gambian infants. Correction of title from Randomised trial of Haemophilus influenzae type-b tetanus protein **conjugate** for prevention of pneumonia and meningitis in Gambian infants. Erratum published in Lancet (North American Edition) Vol. 350. **Iss**. 9076. 1997. p. 524. Mulholland, Kim (1); Hilton, Stephen; Adegbola, Richard; Usen, Stanley; Oparaugo, Anslem; Omosigho, Charles; Weber, Martin; Palmer, Ayo; Schneider, Gisela; Jobe, Kebba; Lahai, George; Jaffar, Shabbar; Secka, Ousman; Lin, Kimi; Ethevenaux, Chantal; Greenwood, Brian. (1) Div. Child. Health Dev., Global Programme of Vaccines Immunisation, World Health Organization, 1211 Geneva 27 Switzerland. The Lancet, (Aug, 1997) Vol. 349, No. 9060, pp. 1191-1197. ISSN: 0099-5355. Language: English.

AB Background: In developing countries, pneumonia and meningitis due to Haemophilus influenzae type b (Hib) are common in children under age 12 months and the mortality from meningitis is high. Protein-polysaccharide **conjugate vaccines** have brought Hib disease under control in industrialized countries. We did a double-blind randomized trial in The Gambia to assess the efficacy of a Hib **conjugate vaccine** for the prevention of meningitis, pneumonia, and other invasive diseases due to Hib. Methods: Between March, 1993, and October, 1995, 42 848 infants were randomly allocated the **conjugate vaccine** Hib polysaccharide tetanus protein (PRP-T) mixed with diphtheria-tetanus-pertussis **vaccine** (DTP), or DTP alone at age 2 months, 3 months, and 4 months. Children who presented with signs of invasive Hib were investigated by blood culture and, where appropriate, by lumbar puncture, chest radiograph, or percutaneous lung aspirate. Children were followed up for between 5 and 36 months. Findings: The median ages at which children received the study **vaccine** were 11 weeks, 18 weeks, and 24 weeks. 83% of children enrolled received all three doses of **vaccine**. 17 cases of culture-positive Hib pneumonia, 28 of Hib meningitis, and five of other forms of invasive Hib disease were detected amongst the study children. The efficacy of the **vaccine** for the prevention of all invasive disease after three doses was 95% (PRP-T vaccinees 1, controls 19 (95% CI 67-100)), for the prevention of Hib pneumonia after two or three doses, 100% (vaccinees 0, controls 10 (55-100)), and for the prevention of radiologically defined pneumonia at any time after enrolment, 21.1% (PRP-T vaccinees 198, controls 251 (4.6-34.9)). Interpretation: PRP-T **conjugate** Hib **vaccine** prevented most cases of meningitis and pneumonia due to Hib in Gambian infants. The reduction in the overall incidence of radiologically defined pneumonia in PRP-T vaccinees suggests that about 20% of episodes of pneumonia in young Gambian children are due to Hib. The introduction of Hib **vaccines** into developing countries should substantially reduce childhood mortality due to pneumonia and meningitis.

L13 ANSWER 9 OF 10 BIOSIS COPYRIGHT 2001 BIOSIS
1998:121757 Document No.: PREV199800121757. Correction of PREVIEWS 99472569. Immunogenicity and safety of a liquid combination of DTP-PRP-T vs lyophilized PRP-T reconstituted with DTP. Correction of title from Immunogenicity and safety of a liquid combination of DT-PRP-T vs lyophilized PRP-T reconstituted with DTP. Erratum published in **Vaccine** Vol. 15. **Iss**. 16. 1997. p. 1813. Amir, Jacob;

Melamed, Rimma; Bader, Juma; Ethevenaux, Chantal; Fritzell, Bernard; Cartier, Jean R.; Arminjon, Francois; Dagan, Ron (1). (1) Pediatr. Infect. Dis. Unit, Ben Gurion Univ. of the Negev, Soroka Univ. Med. Cent., Fac. Health Sci., Beer-Sheva 84101 Israel. Vaccine, (1997) Vol. 15, No. 16, pp. 149-154. ISSN: 0264-410X. Language: English.

AB The immunogenicity and safety of a combined diphtheria, tetanus, pertussis and Haemophilus influenzae type b-tetanus **conjugate vaccine** (DTP-PRP-T) was compared to the same combination obtained by the reconstitution of H. influenzae type b-tetanus **conjugate vaccine** lyophilized (PRP-T) with liquid diphtheria-tetanus-pertussis **vaccine** (DTP). Two hundred and sixty-two healthy infants were randomized to receive a intramuscular injection of 0.5 ml of one of the above combination **vaccines** at 2, 4 and 6 months of age, and a subgroup of 134 infants received a booster dose at 12 months. Serum antibody levels to each **vaccine** component were measured at ages 2, 6, 7, 12 and 13 months. Systemic and local reactions were assessed during the first 3 days after each injection by diary cards distributed to the parents. After the third dose and booster administered at 12 months of age, significant equivalence between the groups was observed, and the geometric mean titers of anti H. influenzae type b capsular polysaccharide (Hib-CP) antibodies were 5.9 and 32.6 mug ml-1 for the liquid combination group and 5.8 and 19.4 for the lyophilized group, respectively. After the third dose, anti-Hib-PC antibody levels of gtoreq1.0 mug ml-1 and 0.15 mug ml-1 were seen in 94% and 100%, respectively, of the liquid combination group and 90 and 99%, respectively of the lyophilized group. After the booster dose, levels of gtoreq1.0 mug/ml-1 were observed in 100% and 93.5% of the liquid combination group and the lyophilized combination group, respectively. Systemic and local reactions to the vaccination were generally mild and did not differ significantly between the groups. We conclude that the liquid combination of DTP-PRP-T is safe and at least as immunogenic as the lyophilized preparation. This liquid preparation, like other combined **vaccines** may be helpful for planning vaccination programs with a reduced number of injections.

L13 ANSWER 10 OF 10 BIOSIS COPYRIGHT 2001 BIOSIS DUPLICATE 7

1991:318707 Document No.: BA92:29222. HETEROTYPIC PASSIVE PROTECTION INDUCED BY SYNTHETIC PEPTIDES CORRESPONDING TO VP7 AND VP4 OF BOVINE ROTAVIRUS. ADDITION OF AUTHOR NAME. ERRATUM PUBLISHED IN J VIROL VOL. 65. **ISS** . 9. 1991. P. 5130. IJAZ M K; ATTAH-POKU S K; REDMOND M J; PARKER M D; SABARA M I; FRENCHICK P; BABIUK L A. VET. INFECT. DIS. ORGANIZATION, WESTERN COLL. VET. MED., UNIV. SASK., SASKATOON, SASK. S7N 0W0, CAN.. J VIROL, (1991) 65 (6), 3106-3113. CODEN: JOVIAM. ISSN: 0022-538X. Language: English.

AB We have evaluated the potential of two peptides derived from highly conserved regions of rotavirus outer capsid proteins (VP7 and VP4) to act as a rotavirus **vaccine**. The capacity of peptides coupled to rotavirus VP6 spherical particles to provide passive protection in a murine model was compared with the protection induced by peptide-keyhole limpet hemocyanin (KLH) **conjugates**. Female mice were immunized a total of three times before and during pregnancy. Suckling mouse pups were challenged at 7 days of age with either homologous or heterologous rotavirus serotypes. The efficacy of vaccination was determined by analyzing the clinical symptoms and measuring xylose adsorption in the

intestine. In this model the VP4 peptide-VP6 **conjugate** provided protection equal to that obtained using bovine rotavirus (BRV) as the immunogen. The VP7 peptide-VP6 **conjugate** provided slightly less protection than the VP4 peptide-VP6 **conjugate**. A mixture of the VP4 peptide-VP6 and VP7 peptide-VP6 **conjugates** provided better heterologous protection than immunization with BRV. In contrast, KLH-conjugated peptides provided only partial protection. The significance of a synthetic-peptide-based rotavirus **vaccine** in the prevention of infections is discussed.

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FILE 'MEDLINE, EMBASE, BIOSIS, SCISEARCH, CAPLUS' ENTERED AT 15:33:43 ON 09 OCT 2001

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L1          378 S TUCK S?/AU OR NEST G?/AU
L2            0 S L1 AND IMMUNOSTIMULATORY SEUQENCE
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L5            6 DUP REMOVE L4 (8 DUPLICATES REMOVED)
L6         9312 S "ISS"
L7            0 S L5 AND IMMUNOSTIMULATORY SEUQENCE
L8            8 S L6 AND POLYNUCLEOTIDE
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L13          10 DUP REMOVE L11 (19 DUPLICATES REMOVED)
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L14 ANSWER 1 OF 10 MEDLINE DUPLICATE 1

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Dr. E. Raz, Department of Medicine, Univ. of California at San Diego, 9500 Gilman Drive, San Diego, CA 92093-0663, United States. eraz@ucsd.edu.
Journal of Immunology 167/3 (1584-1591) 1 Aug 2001.
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ISSN: 0022-1767. CODEN: JOIMA3. Pub. Country: United States. Language: English. Summary Language: English.
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AB Allergic diseases are a growing health concern in industrialized countries. Despite a number of effective therapeutic options for the prevention and treatment of the pathophysiologic responses which characterize allergic diseases, the induction of true allergen desensitization remains an elusive therapeutic goal. Only immunotherapy (IT) has been shown to have any effect on the underlying hypersensitivities which mediate allergic reactions, and traditional protein-based allergen IT has a limited scope of efficacy However, a number of reagents collectively termed DNA-based immunotherapeutics have proven highly effective in both the prevention and reversal of Th2-mediated hypersensitivity states in mouse models of allergic disease. Four basic DNA-based immunotherapeutic modalities have been used for these studies. These include immunization with gene **vaccines**, allergen mixed with immunostimulatory oligodeoxynucleotide (**ISS**-ODN), and physical allergen-**ISS**-ODN **conjugates** (AIC), as well as immunomodulation with **ISS**-ODN alone. Results from many laboratories have generated guarded optimism that DNA-based immunotherapeutics may be effective for the reversal of allergic hypersensitivity states in humans, and several clinical trials have

already been initiated. This review will focus on our present understanding of the biological activities of DNA-based immunotherapeutics and their application to the treatment of allergic diseases.

L14 ANSWER 4 OF 10 MEDLINE DUPLICATE 4
2000427428 Document Number: 20395232. PubMed ID: 10940883. Conjugation of protein to immunostimulatory DNA results in a rapid, long-lasting and potent induction of cell-mediated and humoral immunity. Tighe H; Takabayashi K; Schwartz D; Marsden R; Beck L; Corbeil J; Richman D D; Eiden J J Jr; Spiegelberg H L; Raz E. (Department of Medicine, The Sam and Rose Stein Institute for Research of Aging, University of California, San Diego, La Jolla 92093-0663, USA.. htighe@ucsd.edu) . EUROPEAN JOURNAL OF IMMUNOLOGY, (2000 Jul) 30 (7) 1939-47. Journal code: EN5; 1273201. ISSN: 0014-2980. Pub. country: GERMANY: Germany, Federal Republic of. Language: English.

AB Immunostimulatory DNA sequences (**ISS**) are a potent Th1 adjuvant. We hypothesized that conjugation of **ISS** to protein antigens would strongly enhance their immunogenicity because both antigen and adjuvant (**ISS**) would be delivered to the same locale/antigen-presenting cell. To test this hypothesis, we conjugated a 22-mer immunostimulatory oligodeoxynucleotide (**ISS**-ODN) to two test antigens of differing intrinsic immunogenicity, namely Escherichia coli beta-galactosidase and the HIV-1 envelope glycoprotein gp120. We show that the antigen-**ISS conjugates** rapidly induce Th1 cells secreting high levels of IFN-gamma, strong CTL activity, and high titer IgG2a and HIV-neutralizing antibodies, exceeding gene and protein vaccination alone or immunization with mixtures of antigen and **ISS** -ODN. The data suggest that this procedure generates a novel and unique **vaccine** that rapidly triggers strong humoral and cell-mediated immunity.

L14 ANSWER 5 OF 10 MEDLINE DUPLICATE 5
2000264172 Document Number: 20264172. PubMed ID: 10802617. Immunostimulatory DNA-based **vaccines** induce cytotoxic lymphocyte activity by a T-helper cell-independent mechanism. Cho H J; Takabayashi K; Cheng P M; Nguyen M D; Corr M; Tuck S; Raz E. (Department of Internal Medicine and The Sam and Rose Stein Institute for Research on Aging, University of California San Diego, La Jolla, CA 92093-0663, USA.) NATURE BIOTECHNOLOGY, (2000 May) 18 (5) 509-14. Journal code: CQ3; 9604648. ISSN: 1087-0156. Pub. country: United States. Language: English.

AB Immunostimulatory DNA sequences (**ISS**) contain unmethylated CpG dinucleotides within a defined motif. Immunization with **ISS** -based **vaccines** has been shown to induce high antigen-specific cytotoxic lymphocyte (CTL) activity and a Th1-biased immune response. We have developed a novel **ISS**-based **vaccine** composed of ovalbumin (OVA) chemically conjugated to **ISS**-oligodeoxynucleotide (ODN). Protein-**ISS conjugate** (PIC) is more potent in priming CTL activity and Th1-biased immunity than other **ISS**-based **vaccines**. Cytotoxic lymphocyte activation by **ISS**-ODN-based **vaccines** is preserved in both CD4-/- and MHC class II-/- gene-deficient animals. Furthermore, PIC provides protection against a lethal burden of OVA-expressing tumor cells in a CD8+ cell-dependent manner. These results demonstrate that PIC acts through two unique mechanisms: T-helper-independent activation of CTL and facilitation of exogenous antigen presentation on MHC class I. This technology may have clinical applications in cancer therapy and in stimulating host defense in AIDS and chronic immunosuppression.

L14 ANSWER 6 OF 10 MEDLINE DUPLICATE 6
2000166981 Document Number: 20166981. PubMed ID: 10700464. Synthetic oligodeoxynucleotide containing CpG motif induces an anti-polysaccharide type 1-like immune response after immunization of mice with Haemophilus influenzae type b **conjugate vaccine**. von Hunolstein C; Teloni R; Mariotti S; Recchia S; Orefici G; Nisini R. (Laboratorio di Batteriologia e Micologia Medica, Istituto Superiore di Sanita, Viale Regina Elena 299, 00161 Roma, Italy.) INTERNATIONAL IMMUNOLOGY, (2000 Mar) 12 (3) 295-303. Journal code: AY5; 8916182. ISSN: 0953-8178. Pub. country: ENGLAND: United Kingdom. Language: English.

AB Synthetic oligodeoxynucleotides containing CpG motifs [immunostimulatory sequences (**ISS**)] have been described as potent adjuvants of type 1 immune responses when co-administered with protein or peptide **vaccines**. To investigate their role in the immune response to polysaccharides (CHO), different preparations of anti-Haemophilus influenzae type b (Hib) **conjugate vaccine** were administered to mice. The unconjugated CHO did not induce the synthesis of specific antibodies even in the presence of **ISS**. On the other hand, anti-CHO-specific antibodies significantly increased in the presence of **ISS**, when tetanus (TT) or diphtheria [cross-reacting material (CRM)] toxoid-conjugated CHO were used to immunize mice. The adjuvant effect was also observed for the immune response against the carrier protein (TT and CRM). **ISS** insured an early and long-lasting specific IgG production. The effects of **ISS** on the anti-CHO immune response could be attributed to the amplification of the T help provided by the carrier. The analysis of anti-CHO IgG subclasses showed a significant increase of IgG2a and IgG3 in the presence of **ISS**. **ISS** caused a rapid release of IL-12 and IFN-gamma in sera from treated mice. This data provide a first evidence for the ability of **ISS** to induce an anti-CHO type 1-like immune response and demonstrate that **ISS** have the potential to increase host antibody response against both the CHO and the protein component of a conjugated **vaccine**.

L14 ANSWER 7 OF 10 MEDLINE
2000387434 Document Number: 20347064. PubMed ID: 10887315. Conjugation of immunostimulatory DNA to the short ragweed allergen amb a 1 enhances its immunogenicity and reduces its allergenicity. Tighe H; Takabayashi K; Schwartz D; Van Nest G; Tuck S; Eiden J J; Kagey-Sobotka A; Creticos P S; Lichtenstein L M; Spiegelberg H L; Raz E. (Department of Medicine and The Sam and Rose Stein Institute for Research on Aging, and the Department of Pediatrics, University of California San Diego School of Medicine, La Jolla, CA, USA.) JOURNAL OF ALLERGY AND CLINICAL IMMUNOLOGY, (2000 Jul) 106 (1 Pt 1) 124-34. Journal code: H53; 1275002. ISSN: 0091-6749. Pub. country: United States. Language: English.

AB BACKGROUND: Allergen immunotherapy is inconvenient and associated with the risk of anaphylaxis. Efforts to improve the safety of immunotherapy by means of chemical modification of allergens have not been successful because it greatly reduced their antigenicity. Recently, immunostimulatory DNA sequences (**ISS** or CpG motifs) have been shown to act as strong T(H)1 response-inducing adjuvants. OBJECTIVE: We sought to determine whether conjugation of **ISS** to the major short ragweed allergen Amb a 1 results in enhanced immunotherapeutic potential in mice and decreased allergenicity in human subjects. METHODS: A 22-mer **ISS** oligodeoxynucleotide (**ISS**-ODN) was coupled to Amb a 1 and used for immunization of mice, rabbits, and monkeys. RESULTS: In mice the Amb a 1-**ISS conjugate** induced a T(H)1 response (IFN-gamma secretion), whereas Amb a 1 induced a T(H)2 response (IL-5 secretion). The T(H)1 response was not observed with an Amb a 1-non-

ISS conjugate. Coinjection of Amb a 1 with **ISS**
-ODN was much less effective in inducing a T(H)1 response. In mice primed for a T(H)2 response, injection with Amb a 1-**ISS conjugate** induced a de novo T(H)1 response and suppressed IgE antibody formation after challenge with Amb a 1. Amb a 1-**ISS conjugate** induced high-titer anti-Amb a 1 IgG antibodies in rabbits and cynomolgus monkeys, whereas Amb a 1 alone or Amb a 1 coinjected with **ISS**-ODN did not induce a detectable response. Amb a 1-**ISS conjugate** was less allergenic than Amb a 1 alone, as shown by a 30-fold lower histamine release from human basophils of patients with ragweed allergy, whereas mixing **ISS**-ODN with Amb a 1 did not reduce histamine release. CONCLUSION: Amb a 1-**ISS conjugate** has an enhanced T(H)1-biased immunogenicity and reduced allergenicity. It may offer a more effective and safer approach for allergen immunotherapy than currently available methods.

L14 ANSWER 8 OF 10 BIOSIS COPYRIGHT 2001 BIOSIS
1998:98255 Document No.: PREV199800098255. Correction of PREVIEWS 99554579. Randomised trial of Haemophilus influenzae type-b tetanus protein **conjugate vaccine** for prevention of pneumonia and meningitis in Gambian infants. Correction of title from Randomised trial of Haemophilus influenzae type-b tetanus protein **conjugate** for prevention of pneumonia and meningitis in Gambian infants. Erratum published in Lancet (North American Edition) Vol. 350. **Iss**. 9076. 1997. p. 524. Mulholland, Kim (1); Hilton, Stephen; Adegbola, Richard; Usen, Stanley; Oparaugo, Anslem; Omosigho, Charles; Weber, Martin; Palmer, Ayo; Schneider, Gisela; Jobe, Kebba; Lahai, George; Jaffar, Shabbar; Secka, Ousman; Lin, Kimi; Ethevenaux, Chantal; Greenwood, Brian. (1) Div. Child. Health Dev., Global Programme of Vaccines Immunisation, World Health Organization, 1211 Geneva 27 Switzerland. The Lancet, (Aug, 1997) Vol. 349, No. 9060, pp. 1191-1197. ISSN: 0099-5355. Language: English.

AB Background: In developing countries, pneumonia and meningitis due to Haemophilus influenzae type b (Hib) are common in children under age 12 months and the mortality from meningitis is high. Protein-polysaccharide **conjugate vaccines** have brought Hib disease under control in industrialized countries. We did a double-blind randomized trial in The Gambia to assess the efficacy of a Hib **conjugate vaccine** for the prevention of meningitis, pneumonia, and other invasive diseases due to Hib. Methods: Between March, 1993, and October, 1995, 42 848 infants were randomly allocated the **conjugate vaccine** Hib polysaccharide tetanus protein (PRP-T) mixed with diphtheria-tetanus-pertussis **vaccine** (DTP), or DTP alone at age 2 months, 3 months, and 4 months. Children who presented with signs of invasive Hib were investigated by blood culture and, where appropriate, by lumbar puncture, chest radiograph, or percutaneous lung aspirate. Children were followed up for between 5 and 36 months. Findings: The median ages at which children received the study **vaccine** were 11 weeks, 18 weeks, and 24 weeks. 83% of children enrolled received all three doses of **vaccine**. 17 cases of culture-positive Hib pneumonia, 28 of Hib meningitis, and five of other forms of invasive Hib disease were detected amongst the study children. The efficacy of the **vaccine** for the prevention of all invasive disease after three doses was 95% (PRP-T vaccinees 1, controls 19 (95% CI 67-100)), for the prevention of Hib pneumonia after two or three doses, 100% (vaccinees 0, controls 10 (55-100)), and for the prevention of radiologically defined pneumonia at any time after enrolment, 21.1% (PRP-T vaccinees 198, controls 251 (4.6-34.9)). Interpretation: PRP-T **conjugate** Hib **vaccine** prevented most cases of meningitis and pneumonia due to Hib in Gambian infants. The reduction in the overall incidence of radiologically defined

pneumonia in PRP-T vaccinees suggests that about 20% of episodes of pneumonia in young Gambian children are due to Hib. The introduction of Hib **vaccines** into developing countries should substantially reduce childhood mortality due to pneumonia and meningitis.

L14 ANSWER 9 OF 10 BIOSIS COPYRIGHT 2001 BIOSIS
1998:121757 Document No.: PREV199800121757. Correction of PREVIEWS 99472569. Immunogenicity and safety of a liquid combination of DTP-PRP-T vs lyophilized PRP-T reconstituted with DTP. Correction of title from Immunogenicity and safety of a liquid combination of DT-PRP-T vs lyophilized PRP-T reconstituted with DTP. Erratum published in **Vaccine** Vol. 15. **Iss**. 16. 1997. p. 1813. Amir, Jacob; Melamed, Rimma; Bader, Juma; Ethevenaux, Chantal; Fritzell, Bernard; Cartier, Jean R.; Arminjon, Francois; Dagan, Ron (1). (1) Pediatr. Infect. Dis. Unit, Ben Gurion Univ. of the Negev, Soroka Univ. Med. Cent., Fac. Health Sci., Beer-Sheva 84101 Israel. Vaccine, (1997) Vol. 15, No. 16, pp. 149-154. ISSN: 0264-410X. Language: English.
AB The immunogenicity and safety of a combined diphtheria, tetanus, pertussis and Haemophilus influenzae type b-tetanus **conjugate** **vaccine** (DTP-PRP-T) was compared to the same combination obtained by the reconstitution of H. influenzae type b-tetanus **conjugate** **vaccine** lyophilized (PRP-T) with liquid diphtheria-tetanus-pertussis **vaccine** (DTP). Two hundred and sixty-two healthy infants were randomized to receive a intramuscular injection of 0.5 ml of one of the above combination **vaccines** at 2, 4 and 6 months of age, and a subgroup of 134 infants received a booster dose at 12 months. Serum antibody levels to each **vaccine** component were measured at ages 2, 6, 7, 12 and 13 months. Systemic and local reactions were assessed during the first 3 days after each injection by diary cards distributed to the parents. After the third dose and booster administered at 12 months of age, significant equivalence between the groups was observed, and the geometric mean titers of anti H. influenzae type b capsular polysaccharide (Hib-CP) antibodies were 5.9 and 32.6 mug ml-1 for the liquid combination group and 5.8 and 19.4 for the lyophilized group, respectively. After the third dose, anti-Hib-PC antibody levels of gtoreq1.0 mug ml-1 and 0.15 mug ml-1 were seen in 94% and 100%, respectively, of the liquid combination group and 90 and 99%, respectively of the lyophilized group. After the booster dose, levels of gtoreq1.0 mug/ml-1 were observed in 100% and 93.5% of the liquid combination group and the lyophilized combination group, respectively. Systemic and local reactions to the vaccination were generally mild and did not differ significantly between the groups. We conclude that the liquid combination of DTP-PRP-T is safe and at least as immunogenic as the lyophilized preparation. This liquid preparation, like other combined **vaccines** may be helpful for planning vaccination programs with a reduced number of injections.

L14 ANSWER 10 OF 10 BIOSIS COPYRIGHT 2001 BIOSIS DUPLICATE 7
1991:318707 Document No.: BA92:29222. HETEROTYPIC PASSIVE PROTECTION INDUCED BY SYNTHETIC PEPTIDES CORRESPONDING TO VP7 AND VP4 OF BOVINE ROTAVIRUS. ADDITION OF AUTHOR NAME. ERRATUM PUBLISHED IN J VIROL VOL. 65. **ISS**. 9. 1991. P. 5130. IJAZ M K; ATTAH-POKU S K; REDMOND M J; PARKER M D; SABARA M I; FRENCHICK P; BABIUK L A. VET. INFECT. DIS. ORGANIZATION, WESTERN COLL. VET. MED., UNIV. SASK., SASKATOON, SASK. S7N 0W0, CAN.. J VIROL, (1991) 65 (6), 3106-3113. CODEN: JOVIAM. ISSN: 0022-538X. Language:

English.

AB We have evaluated the potential of two peptides derived from highly conserved regions of rotavirus outer capsid proteins (VP7 and VP4) to act as a rotavirus **vaccine**. The capacity of peptides coupled to rotavirus VP6 spherical particles to provide passive protection in a murine model was compared with the protection induced by peptide-keyhole limpet hemocyanin (KLH) **conjugates**. Female mice were immunized a total of three times before and during pregnancy. Suckling mouse pups were challenged at 7 days of age with either homologous or heterologous rotavirus serotypes. The efficacy of vaccination was determined by analyzing the clinical symptoms and measuring xylose adsorption in the intestine. In this model the VP4 peptide-VP6 **conjugate** provided protection equal to that obtained using bovine rotavirus (BRV) as the immunogen. The VP7 peptide-VP6 **conjugate** provided slightly less protection than the VP4 peptide-VP6 **conjugate**. A mixture of the VP4 peptide-VP6 and VP7 peptide-VP6 **conjugates** provided better heterologous protection than immunization with BRV. In contrast, KLH-conjugated peptides provided only partial protection. The significance of a synthetic-peptide-based rotavirus **vaccine** in the prevention of infections is discussed.

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Vol. 266, No. 2, Issue of January 15, pp. 1229-1236, 1991
Printed in U.S.A.

Cloning of *Amb a I* (Antigen E), the Major Allergen Family of Short Ragw ed Poll n

(Received for publication, June 22, 1990)

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To determine the structure of *Amb a I* (previously called antigen E), the major allergen from short ragweed, cDNA from pollen was cloned into λgt11 and λgt10. One of the three distinct clones isolated from the λgt11 library by screening with anti-denatured *Amb a I* antibodies was used to screen both libraries for other *Amb a I* sequences. Multiple clones were isolated and sequenced and proved to be highly homologous but nonidentical. The clones could be divided into three groups based on sequence similarity, and in accordance with the International Union of Immunological Societies-approved nomenclature (Marsh, D. G., Goodfriend, L., King, T. P., Lowenstein, H., and Platts-Mills, T. A. E. (1986) *Bull. WHO* 64, 767–770) they have been designated *Amb a I.1*, *Amb a I.2*, and *Amb a I.3*. Clones within a group have greater than 99% identity, and similarity among groups is 85–90% at the nucleotide level. The amino acid sequence of four peptides (isolated from antigen E obtained from the Research Resources Branch of the National Institutes of Health) containing 132 amino acids was identical to one of the clones (*Amb a I.1*). The presence of multiple naturally occurring isoelectric forms of *Amb a I* was demonstrated by two-dimensional gel electrophoresis and Western blotting. Southern blot analysis demonstrates the presence of multiple *Amb a I*-related sequences in the ragweed genome. *Amb a I* is therefore not a single molecule but rather a family of closely related proteins.

Of all the seasonal aeroallergens, pollen from short ragweed (*Ambrosia artemisiifolia*) is perhaps the most clinically important. The extremely small particle size allows this pollen to be carried hundreds of miles, and it is the major cause of late summer hay fever in the eastern United States and Canada (2). Of the 52 antigens present in an aqueous extract of pollen, at least 22 are allergens defined by their reactivity with human IgE (3). At least five significant human allergens from short ragweed pollen have been purified to homogeneity and studied with respect to their biochemical and immunological characteristics (2).

Antigen E or *Amb a I* (according to nomenclature in Ref. 1) is considered the most important allergen since 95% of ragweed-sensitive individuals react to it in skin tests and show high IgE antibody titers to it (4, 5). *Amb a I* is highly abundant, comprising about 6% of the total protein in a neutral aqueous extract of pollen (4). It is an acidic, amino-terminal "blocked," reportedly nonglycosylated single-chain protein of 38 kDa which undergoes proteolysis during chromatographic purification and is cleaved into two chains, α and β, of 26 and 12 kDa, respectively (6). The two-chain form is reported to be allergenically and antigenically indistinguishable from the intact molecule, but modification of the protein, including reduction and alkylation of disulfide bonds, urea denaturation and renaturation, or succinylation of lysine residues, reduces the IgE immunoreactivity of the molecule (2). Recent data with Western blotting demonstrate that *Amb a I* retains the ability to bind antibody from allergic humans and hyperimmunized animals (documented in Fig. 3 and Footnote 1).

It has been reported that immunotherapy utilizing purified *Amb a I* is as effective in alleviating clinical symptoms in allergic patients as is immunotherapy using whole pollen extract (7). Immunizations with modified forms of *Amb a I* have also been tested and shown to be as clinically effective as native *Amb a I* and to cause fewer systemic reactions (8–11). *Amb a I* has been shown to have three nonoverlapping, nonrepeated antigenic sites, as defined by murine monoclonal antibodies, of which at least two represent major human allergenic epitopes (12). In addition, preliminary studies directed at examining T cell epitopes of *Amb a I* suggest that they are linear rather than conformational (13–15).

At present, desensitization immunotherapy for ragweed-allergic individuals relies upon multiple injections of small doses of aqueous pollen extracts. These protocols are not ideal since individuals present with varying sensitivities to each of the multiple components in an extract, and various batches of extract used for diagnostic and therapeutic purposes vary a great deal in their specific allergen content. Furthermore, although immunotherapy offers some improvement to many patients, almost no patients become completely asymptomatic, and a number of patients show no symptomatic improvement at all (16). Identification and characterization of specific epitopes of *Amb a I* might be particularly useful in improving an immunotherapeutic approach to desensitization.

As a first step toward this end, cDNA libraries have been constructed from short ragweed pollen and whole flowers. This report describes the cloning of *Amb a I* and presents the complete nucleotide and deduced amino acid sequences of three clones coding for this 398-amino acid allergen. Comparison of the three cloned sequences shows that they have

* This work was supported by National Institutes of Health Grant AI 14908 (to D. G. K.) and by a grant from the North Carolina Biotechnology Center (to D. G. K.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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[1] T. Rafnar, I. J. Griffith, M-C. Kuo, J. F. Bond, B. L. Rogers, and D. G. Klapper, unpublished results.

several differences, suggesting that *Amb a I* is actually a family of proteins with at least three members. These proteins represent an as yet undescribed family since a search of available data bases shows no significant homology to other proteins from either procaryotes or eucaryotes. Finally, in contrast to what has been reported in the literature (6), the carboxyl terminus of *Amb a I* appears to be cysteine. During this investigation, a peptide corresponding to the predicted carboxyl terminus of *Amb a I* was isolated from chromatographically purified *Amb a I*, confirming the assignment of cysteine as the carboxyl-terminal amino acid.

MATERIALS AND METHODS

Antibodies—Mouse monoclonal anti-denatured *Amb a I* antibodies JB4F3-5, JB3C9-3, JB1E3-4, 2D8/E6, JB4E3-3, JB4E6-4, and IB1E2-2 have been described previously (17).

Plant Tissue—Flowers and leaves of short ragweed were picked, frozen immediately in liquid nitrogen, and stored at −70 °C until processing. Defatted short ragweed pollen and meadow fescue pollen were obtained from Greer Laboratories (Lenoir, NC).

Genomic DNA Isolation—Genomic DNA was isolated by a method published previously (18) with the following changes: 50–100 g of frozen ragweed flowers were ground in liquid nitrogen using a mortar and pestle. The dry powder was suspended in homogenization buffer (0.2 M Tris-HCl, pH 8.5, 0.2 M sucrose, 6 mM KCl, 50 mM $MgCl_2$, 5.8 mM 2-mercaptoethanol, 2% (w/v) polyvinylpyrrolidone 40 and homogenized at high speed with a Polytron homogenizer (Brinkmann Instruments) for 3 min. The DNA was banded two times on CsCl gradients to obtain DNA that could be digested by restriction enzymes (21).

RNA Isolation—RNA was isolated from 10 g of defatted ragweed pollen or whole flowers by a slightly modified method published by Lagrimini *et al.* (19). After the original phase separation, the nucleic acids were precipitated in the presence of 0.3 M sodium acetate and 75% ethanol at −20 °C for at least 4 h. The precipitate was pelleted by centrifugation at 13,000 × *g* for 20 min and the pellet dissolved in 2 ml of sterile water. The RNA was then precipitated selectively in the presence of 3 M LiCl at −20 °C overnight. The precipitate was pelleted as before.

The RNA pellet was dissolved in 1 ml of oligo(dT) binding buffer (10 mM Tris-HCl, pH 7.5, 0.5 M NaCl, 0.5% SDS,[2] 1 mM EDTA) and poly(A^+) RNA selected on oligo(dT)-cellulose (Collaborative Research). 60 μg of hydrated oligo(dT)-cellulose was added to the sample, heated to 55 °C for 1 min, and mixed gently at room temperature for 10 min. The resin was centrifuged for 5 s in a microcentrifuge, the supernatant poured off, and the sample washed three times with fresh binding buffer before being eluted with 2 × 300 μl of elution buffer (10 mM Tris, pH 7.5, 0.5% SDS, 1 mM EDTA). The poly(A^+) RNA was precipitated with 0.01 volume of 3 M sodium acetate and 2.5 volumes of ethanol.

Isolation and Characterization of cDNA Clones—A λgt11 library was generated from cDNA made from pollen mRNA using avian myeloblastosis virus reverse transcriptase (Life Sciences) and RNase H (Boehringer Mannheim) as described (20). After methylation with *Eco*RI methylase (Bethesda Research Laboratories), cDNA was ligated with phosphorylated *Eco*RI linkers (Bethesda Research Laboratories), digested with *Eco*RI, and excess linker removed from the cDNA on a Sephadex G-100 column (Pharmacia LKB Biotechnology Inc.). The cDNA was ligated into dephosphorylated λgt11 arms (Promega Biotech, Madison, WI), packaged with the Gigapack packaging system (Stratagene Cloning Systems), and plated using standard procedures (21). The library was plated on 150-mm Petri dishes at a density of 20,000–30,000 plaques/plate and screened with a pool of monoclonal mouse anti-*Amb a I* antibodies using the Protoblot kit (Promega Biotech). Antibodies from ascites fluids were prepared by preabsorption with filters of nonrecombinant λ plaque lifts and used at a dilution of 1:5000. Three antibody-binding clones were purified by repeated plating (two to four times), subcloned into M13mp19, and sequenced using the dideoxy sequencing method (22). One of these clones, *Amb a I.2*, was radiolabeled and used to rescreen the library for related nucleotide sequences. This screening yielded six independent additional clones.

A λgt10 library containing cDNA inserts from short ragweed flowerhead mRNA was prepared according to published procedures (23, 24) using an Amersham Corp. cDNA cloning kit. Approximately 8 × 10^5 independent clones were screened by standard procedures (25) using nick-translated (26) ^{32}P-labeled *Amb a I.2* cDNA as a probe. Four independent hybridizing cDNA clones were isolated and subcloned into M13 for dideoxynucleotide sequencing (22).

Northern Blotting and Hybridizations—20 μg of total RNA was electrophoresed through a formaldehyde-containing gel, blotted onto nitrocellulose paper, and hybridized to a radiolabeled cDNA clone according to standard procedures (21). Posthybridization washes were done in 2 × SSC, 0.1% SDS, first at room temperature for 15 min, and then for 1 h at 50 °C. The filter was air dried and exposed to Kodak XAR film at −70 °C with an intensifying screen.

Southern Blot Analysis—Digestion of genomic DNA, agarose gel electrophoresis, and blotting onto nitrocellulose were done by conventional methods (21). The filters were hybridized to nick-translated cDNA clones. Hybridization and washing conditions were the same as those used for RNA blots.

Protein Purification and Sequencing—*Amb a I* protein sequence data were obtained from one chromatographic form of *Amb a I* (AgE-B) generously provided by Dr. T. P. King (Rockefeller University, New York) and from the *Amb a I* protein, which was purified according to the method of King *et al.* (6) with certain modifications. In brief, the defatted short ragweed pollen (Greer Laboratories) was extracted in 50 mM Tris, pH 8.0, containing 0.1 mM phenylmethylsulfonyl fluoride, 1 μg/ml leupeptin, 10 μg/ml pepstatin, and 10 μg/ml soybean trypsin inhibitor. After decolorization with DE52 cellulose (Whatman) the extract was fractionated by ammonium sulfate precipitation. The 45–59% saturation fraction was purified further by a Sephacryl S-200 (Pharmacia) column and Mono Q (Pharmacia) anion-exchange column. The *Amb a I* protein purified by this modified method appeared as a single band of 38 kDa on SDS-polyacrylamide gel electrophoresis (SDS-PAGE). The majority of amino acid sequence data was derived from mixtures of peptides produced either by cyanogen bromide (CNBr) or formic acid (29) cleavage of the AgE-B or *Amb a I*. The peptide mixtures were treated in the sequenator with *o*-phthalaldehyde to block all primary amines when a proline was known to be present at the amino terminus (30), which was identified in an exploratory analysis of the peptide mixtures. One of the peptide mixtures derived from a CNBr digest and an aliquot of AgE-B was subjected to SDS-PAGE. The SDS-PAGE was run on a precast 10–20% Tricine minigel (Novex) run at 100 volts for 1.5 h. Proteins were transferred to polyvinylidene difluoride membrane (Millipore) in 10 mM CAPS buffer, pH 11, at 150 mA for 2 h (27). The polyvinylidene difluoride was stained with Coomassie Blue. The 12-kDa β chain band from AgE-B (6) and a 10-kDa band from the CNBr digest were cut out for protein sequencing. Protein sequencing was performed on an Applied Biosystems model 477A gas-phase sequenator with on-line phenylthiohydantoin derivative analysis (model 120). The protein was alkylated *in situ* in the sequenator by using the nonnucleophilic reductant, tributylphosphine, with concomitant alkylation by 4-vinylpyridine in ethylmorpholine buffer (28).

Two-dimensional Western Blot—Isoelectric focusing was performed on a Hoefer gel apparatus with 15 μg of crude soluble pollen protein. The gel consisted of 7.5% acrylamide with 3.5% Pharmalytes, pH 4.5–5.3 (Pharmacia) and 3.5% Ampholines, pH 3.5–10 (LKB), run at 13 watts for 3.5 h until a constant voltage was reached (31). The gel section was placed on a slab of 10% acrylamide SDS-PAGE and electrophoresed for 3.5 h at 40 mA according to the protocol cited (32). The proteins were transferred overnight in phosphate buffer (20 mM $NaPO_4/NaH_2PO_4$, pH 6.8) to 0.1-μm nitrocellulose (Schleicher & Schuell) at 0.2 A (33). The blot was rinsed in blot solution (25 mM Tris-HCl, pH 7.5, 0.171 M NaCl, 0.05% Tween 20; Sigma). The first antibody incubation was overnight at room temperature with a 1:1000 dilution of goat anti-*Amb a I* IgG in blot solution. The excess first antibody was removed with three 15-min rinses with blot solution. The second antibody was a 1:2500 dilution of biotinylated swine anti-goat IgG (Boehringer Mannheim) in blot solution for 2 h. The blot was then rinsed with blot solution three times (15 min each) and incubated for 1 h in blot solution with ^{125}I-streptavidin (2 μCi, Amersham Corp.). The blot was rinsed with blot solution until the waste wash returned to background. The blot was then exposed to film at −80 °C overnight.

Sequence-specific Amplification of Amb a I cDNA with PCR—4 g of defatted pollen (Greer Laboratories) was ground with a mortar and

[2] The abbreviations used are: SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis; Tricine, *N*-[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]glycine; CAPS, 3-[cyclohexylamino]-1-propanesulfonic acid; PCR, polymerase chain reaction.

pestle in 4 M guanidinium isothiocyanate buffer and total RNA isolated by standard procedures (34).

Priming oligonucleotides (Table I) for DNA amplification (35) were RW38, RW32, RW45, and anchor primer. RW38 corresponded to the amino-terminal coding strand sequence encoding amino acids Leu-Tyr-Phe-Thr-Leu (amino acids 10–14). The RW38 sequence was conserved between *Amb a I.2* and *Amb a I.3*. RW32, which corresponded to the noncoding strand sequence between 12 and 39 nucleotides 3′ of the TAA stop codon, was specific to *Amb a I.1*. RW45 corresponded to the noncoding strand sequence complementary to amino acids Ile-Lys-Ser-Asn-Asp-Gly (amino acids 181–186) of *Amb a I*. Two additional primers, anchor template and anchor linker, were used for linking to cDNA. The anchor primer oligomer sequence was contained within the anchor template sequence. The anchor linker oligomer was phosphorylated (see Table I for specific sequences and restriction sites). These oligonucleotides were purchased from Research Genetics (Huntsville, AL).

First-strand cDNA was synthesized from 1 μg of total RNA with the cDNA Synthesis System Plus kit (Amersham Corp.) using poly(dT) as a primer. The single-stranded DNA (20 μl) was mixed with 100 pmol of each priming oligo, RW38 and RW32, 10 μl of 10 × reaction buffer (GeneAmp kit, U. S. Biochemicals), and 0.5 μl of *Thermus aquaticus* polymerase (U. S. Biochemicals). The mixture was brought to 100 μl with distilled water and overlayed with mineral oil (Sigma). The sample was amplified with a programmable thermal controller from MJ Research, Inc. (Carobridge, MA). The first five rounds of amplification consisted of denaturation at 94 °C for 1 min, annealing of primers to the template at 45 °C for 1.5 min, and chain elongation at 70 °C for 4 min. The final 20 rounds of amplification consisted of denaturation as above, annealing at 55 °C for 1.5 min, and elongation as above.

Amplified DNA was recovered by sequential chloroform, phenol and chloroform extractions followed by overnight precipitation at 4 °C with 0.5 volume of 7.5 M ammonium acetate and 1.5 volumes of isopropyl alcohol. DNA was digested simultaneously with *Eco*RI and *Pst*I and electrophoresed on a 1% GTG agarose (FMC, Rockland, ME) preparative gel. The predicted 1.2-kilobase band was isolated and recovered by glass bead adherence (Geneclean kit, BI0101, La Jolla, CA). The digested DNA was ligated into *Eco*RI/*Pst*I-digested M13 for dideoxy sequencing (22).

The very 5′ end sequence of *Amb a I* was determined using a modification of the anchored PCR (36). Double-stranded cDNA was synthesized from 1 μg of RNA with the cDNA Synthesis System Plus kit using poly(dT) as a primer, blunt ended with T4 polymerase, and blunt end ligated to self-annealed anchor template and anchor linker primers. Linked cDNA (3 μl) was mixed with 100 pmol of the anchor primer and RW32 primers, 10 μl of 10 × reaction buffer and 0.5 μl of *T. aquaticus* polymerase. The mixture was brought to 100 μl and amplified as described above. 1% of the volume of the primary PCR was reamplified with anchor primer and RW45 oligomers. RW45 is nested (internal) relative to oligomer RW32 used in the primary PCR. Amplified DNA from the secondary PCR corresponding to the 5′ end RW45 sequence was recovered as above, digested with *Kpn*I, and ligated into *Kpn*I/*Hin*cII-digested M15 for dideoxy sequencing (22).

RESULTS

Three cl nes were isolated from a λgt11 ragweed pollen cDNA library by screening with a pool of seven mouse monoclonal antibodies raised to denatured *Amb a I*. The sequences f all three f these clones showed extensive homology to peptides isolated from highly purified *Amb a I*. One of these cl nes (*Amb a I.2*) was radiolabeled and used to screen this library and a λgt10 library constructed from cDNA made from whole ragweed flowers, resulting in the isolation of 10 additional clones.

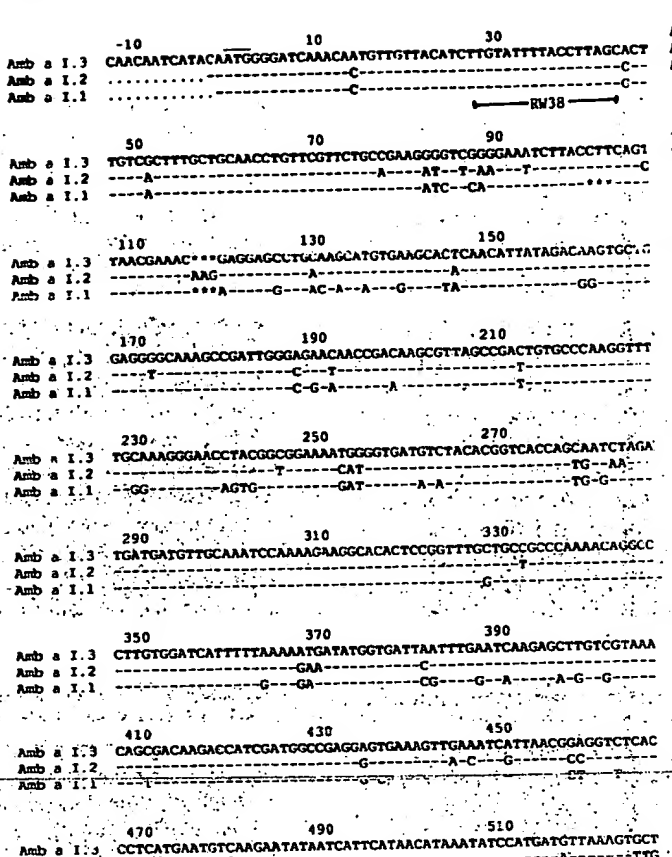
All the clones sequenced could be divided into three groups, *Amb a I.1*, *Amb a I.2*, and *Amb a I.3*, in which clones within a group share greater than 99% identity, and identity among groups ranges from 85 to 90% at the nucleotide level. The DNA sequences of the three largest prototypic clones in each group are shown in Fig. 1. The longest clone, a member of the *Amb a I.3* group, is 1331 bases long, has 13 nucleotides preceding the putative ATG start codon at position 1, an unbroken reading frame of 1190 nucleotides ending with a *TAA* stop codon, and a 125-nucleotide untranslated AT-rich region before the poly(A) addition site. The other clones start at positions −2 and 44 relative to the *Amb a I.3* prototype start site and have unbroken reading frames extending to a stop site identical to that found in the *Amb a I.3* group. The traditional mammalian consensus sequence for polyadenylation, AATAAA, does not occur in the untranslated regions. The *Amb a I.2* group has a nucleotide triplet at position 118 which is not present in the *Amb a I.3* and *Amb a I.1* groups, and the *Amb a I.1* group lacks an additional triplet at position 101 relative to the other two groups of clones. Since only a single non-full-length clone representing the *Amb a I.1* group was originally isolated from the libraries, the full-length clone was obtained by sequence-specific amplifications of pollen RNA using Taq polymerase and synthetic DNA primers. The amino-terminal primer corresponded to coding sequence nucleotides 25–44 in clone *Amb a I.3* and was conserved between the *Amb a I.2* and *Amb a I.3* groups. The carboxyl-terminal primer was specific to *Amb a I.1* and corresponded to the 3′ noncoding sequence between 12 and 29 nucleotides 3′ of the TAA stop codon in that particular clone. The very 5′ end sequence of *Amb a I.1* was determined using anchored PCR methodology. In this case, the amino-terminal primer corresponded to a synthetic linker sequence. The carboxyl-terminal primer described above was used in a primary amplification whereas a carboxyl-terminal primer corresponding to the noncoding strand sequence for amino acids 181–186 was used in a secondary amplification. A secondary amplification using a nested primer was necessary since the primary amplification

TABLE I

Nucleotide sequence of oligomers used in the PCR

RW32, RW38, and RW45 correspond to the coding or noncoding strand sequence of *Amb a I*. RW32 is *Amb a I.1* specific. Anchor primer (AP), anchor linker (AL), and anchor template (AT) do not correspond to *Amb a I* sequences. Nucleotide numbers correspond to those in Fig. 1. Restriction sites are underlined.

Oligomer	Sequence	Strand	Nucleotides
RW32	5′ GGGCTGCAGTCATTATAAGTGCTTAGT *Pst*I	Noncoding	1211–1228
RW38	5′ GGGAATTCTTGTATTTTACCTTAGC *Eco*RI	Coding	27–43
RW45	5′ ACCATCGTTGGACTTAAT	Noncoding	540–557
AL	5′ p-AATGATCGATGCT *Cla*I		
AP	5′ GGGTCTAGAGGTACCGTCCG *Xba*I *Kpn*I		
AT	5′ GGGTCTAGAGGTACCGTCCGATCGATCATT *Xba*I *Kpn*I *Cla*I		



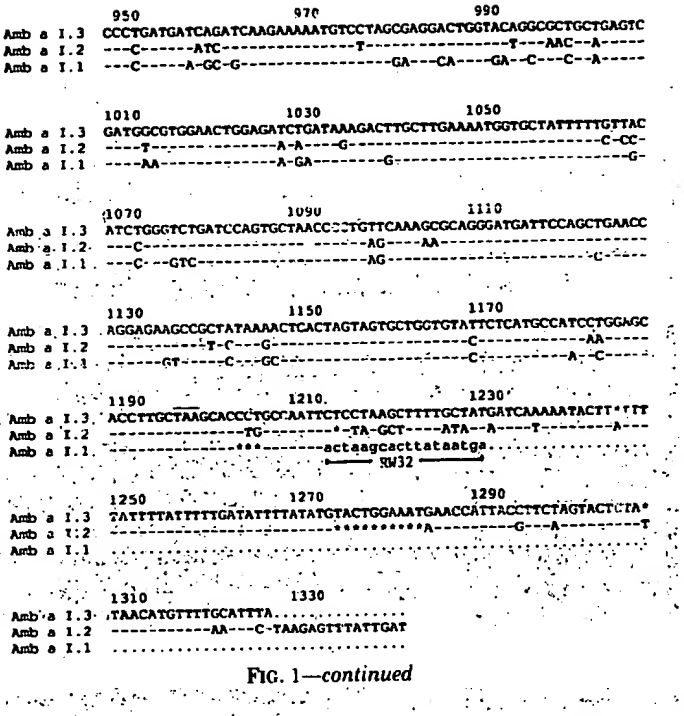
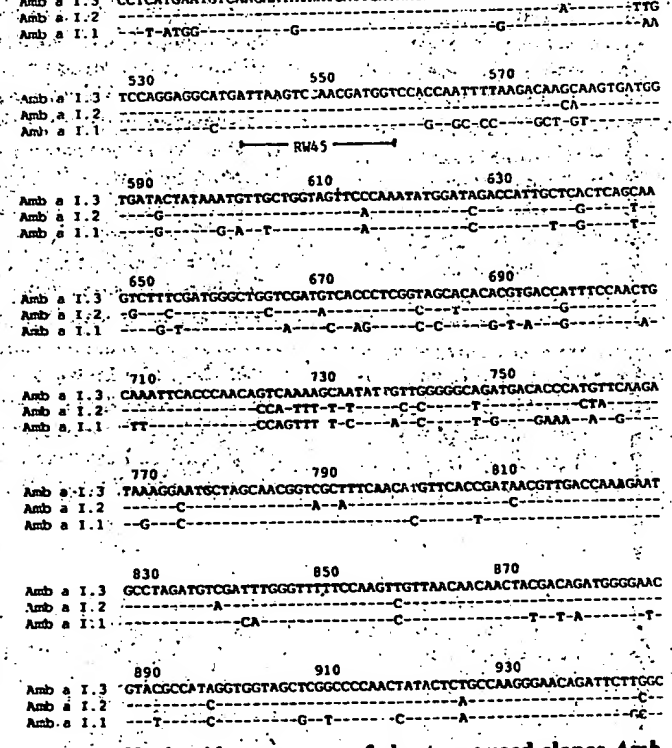


FIG. 1—*continued*



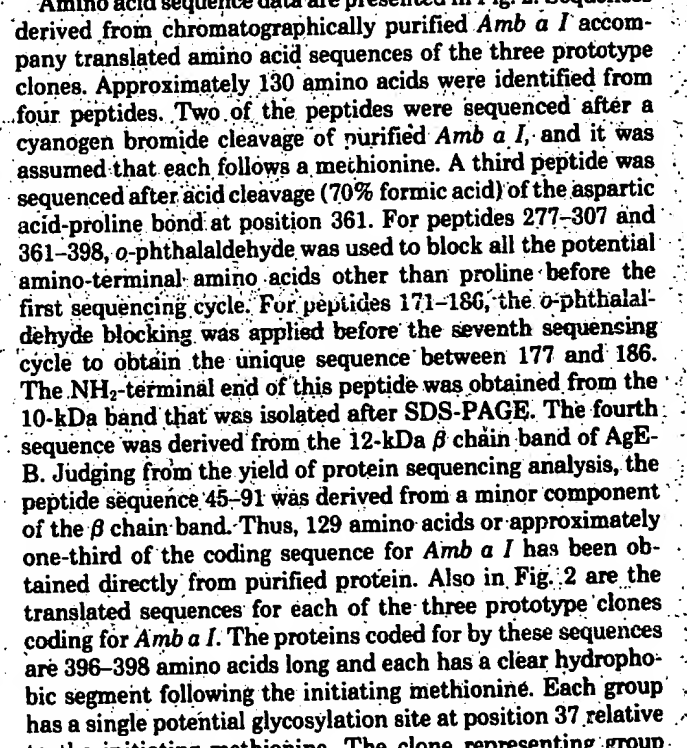


FIG. 1. **Nucleotide sequences of short ragweed clones *Amb a I.3*, *Amb a I.2*, and *Amb a I.1*.** *Numbering* begins with the adenosine of the ATG initiation codon of clone *Amb a I.3*. The start codon and TAA stop codon are *overscored*. *Horizontal bars* (- - -) represent identity to clone *Amb a I.1*; a *dotted line* (. . . .) indicates no sequence information; *asterisks* (*) mark positions at which nucleotides are absent when compared with the other sequences. *Lower case letters* 3′ in the clone *Amb a I.1* indicate the sequence of the 3′ oligonucleotide used for its amplification. The poly(A) tails of the clones are not shown. *Solid lines* (——) indicate the positions of the oligomers used in the PCR.

did not yield a discrete product band. Sequence identities at the nucleotide level in the coding regions are 91% between clones *Amb a I.3* and *Amb a I.2* and 86% between *Amb a I.2* and *Amb a I.1* and between *Amb a I.3* and *Amb a I.1*.

Amino acid sequence data are presented in Fig. 2. Sequences derived from chromatographically purified *Amb a I* accompany translated amino acid sequences of the three prototype clones. Approximately 130 amino acids were identified from four peptides. Two of the peptides were sequenced after a cyanogen bromide cleavage of purified *Amb a I*, and it was assumed that each follows a methionine. A third peptide was sequenced after acid cleavage (70% formic acid) of the aspartic acid-proline bond at position 361. For peptides 277–307 and 361–398, *o*-phthalaldehyde was used to block all the potential amino-terminal amino acids other than proline before the first sequencing cycle. For peptides 171–186, the *o*-phthalaldehyde blocking was applied before the seventh sequensing cycle to obtain the unique sequence between 177 and 186. The NH_2-terminal end of this peptide was obtained from the 10-kDa band that was isolated after SDS-PAGE. The fourth sequence was derived from the 12-kDa β chain band of AgE-B. Judging from the yield of protein sequencing analysis, the peptide sequence 45–91 was derived from a minor component of the β chain band. Thus, 129 amino acids or approximately one-third of the coding sequence for *Amb a I* has been obtained directly from purified protein. Also in Fig. 2 are the translated sequences for each of the three prototype clones coding for *Amb a I*. The proteins coded for by these sequences are 396–398 amino acids long and each has a clear hydrophobic segment following the initiating methionine. Each group has a single potential glycosylation site at position 37 relative to the initiating methionine. The clone representing group *Amb a I.1* corresponds most precisely to known amino acid sequences of peptides derived from enzymatic and chemical cleavages of chromatographically isolated *Amb a I*. The *Amb a I.3* and *Amb a I.2* clones differ by as much as 20% from the known amino acid sequence. The deduced amino acid sequences of clones *Amb a I.3* and *Amb a I.2* are 86% identical t each other (92% similarity), and each has approximately 76% identity (85% similarity) to clone *Amb a I.1*. Nucleotide and amino acid sequence comparisons and calculations were

```
                10                  30                  50
Amb a I.3  MGIKQCCYILYFTLALVALLQPVRSAEGVGEILPSVNETARSLQACEALNIIDKCWRGKA
Amb a I.2  ----H----------T---------D-E-F---A---R---K----H-------C--
Amb a I.1  ----H----------T---------DLQ-----*----*-R-TTSG-Y----C  ----
  PEPTIDE  ..................................................SG-Y----G------

                70                  90                  110
Amb a I.3  DWENNRQALADCAQGFAKGTYGGKWGDVYTVTSNLDDDVANPKEGTLRFAAAQNRPLWII
Amb a I.2  --A--------------------H--------DK-----------------------------
Amb a I.1  --AE--K---------G---V---D--I-----E--------------G---------
  PEPTIDE  --AE--K---------G---V---D--I-u-..................................

                130                 150                 170
Amb a I.3  FKNDMVINLNQELVVNSDKTIDGRGVKVEIINGGLTLMNVKNIIIRNINIHDVKVLPGGM
Amb a I.2  --RN---H--------------------N-V-A--------------------I--C----
Amb a I.1  -ER----R-DK-H----------A------A-F--NG---V------H-----N---L
  PEPTIDE  ..................................................H-----u---L

                190                 210                 230
Amb a I.3  IKSNDGPPILRQASDGDTINVAGSSQIWIDHCSLSKSFDGLVDVTLGSTHVTISNCKFTQ
Amb a I.2  ----------Q----A---------  --------AS---L-I----S---V-------
Amb a I.1  -------AAP-AG----A-SIS--------------V-----AK--T-RL-V--SL---
  PEPTIDE  --u-u-..................................................

                250                 270                 290
Amb a I.3  QSKAILIGADDTHVQDKGMLATVAFNMFTDNVDQRMPRCRFGFFQVVNNNYDRWGTYAIG
Amb a I.2  HQFVL--------Y---------------H-----------------------------
Amb a I.1  HQFVL-F--G-ENIE-R---------T-----------H----------K--S----
  PEPTIDE  ..................------H----------K--S----

                310                 330                 350
Amb a I.3  GSSAPTILCQGNRFLAPDDQIKKNVLARTGTGAAESHAWNWRSDKDLLENGAIFVTSGSD
Amb a I.2  --------S-----F----I----------N----S----T-R---------LP----
Amb a I.1  --AS----S-----C---ERS-----G-H-EA-----K----TN--V--------A--V-
  PEPTIDE  --AS---..........................................

                370                 390
Amb a I.3  PVLTPVQSAGMIPAEPGEAAIKLTSSAGVFSCHPGAPC
Amb a I.2  -----E-K----------VLR-------L---Q----
Amb a I.1  -----E----------S-LS-------L--Q-----
  PEPTIDE  -----E----------S-LS-------Lu-Q-----
```

FIG. 2. **Comparison of the deduced amino acid sequences of clones *Amb a I.3*, *Amb a I.2*, and *Amb a I.1* peptides.** *Horizontal bars* (– – –) represent identity to clone *Amb a I.3*; a *dotted line* (. . . .) indicates no sequence information. *Asterisks* (*) indicate deletions in the amino acid sequences. Unidentified amino acid residues from the peptide sequencing are reprsented by a *u*.

performed using programs described previously (37, 38).

The presence of multiple naturally occurring forms of *Amb a I* in pollen was confirmed by two-dimensional electrophoresis of pollen protein extract (Fig. 3). Pollen proteins were separated first by charge and then by size, blotted onto nitrocellulose, and probed with a goat anti-*Amb a I* antiserum. Three major and three minor variants of *Amb a I* at 38 kDa could be detected with isoelectric points of approximately 5.2, 4.9, 4.7, 4.3, 5.9, and 5.4 in order of decreasing intensity. The calculated (from amino acid composition) isoelectric point of 5.2 of the clone representing *Amb a I.1*, along with its perfect match to *Amb a I* peptides, might suggest that this clone represents the major variant of the allergen isolated from pollen collected over a wide geographic area. The DNA and protein sequences were compared with sequences in the EMBL and NBRF computer data bases. No significant homologies were found in either case.

Northern blot analysis of RNA from defatted ragweed and meadow fescue (grass) pollens is depicted in Fig. 4. RNA was probed with a radiolabeled member of the *Amb a I.2* group. Strong binding can be seen to a 1.5-kilobase message in the ragweed pollen RNA, and no binding is demonstrable in comparable amounts of RNA isolated from the grass pollen. Similar analyses using RNA derived from other ragweed plant tissues such as leaves and roots also showed no binding to the *Amb a I* probe (data not shown).

A Southern blot of genomic DNA digested with various restriction enzymes and probed with *Amb a I.2* is shown in Fig. 5. The clone binds to multiple bands in all the preparations and, when compared with restriction enzyme maps f the clones, suggests that there are multiple genes encoding *Amb a I* in an outbred population of ragweed plants. Whether

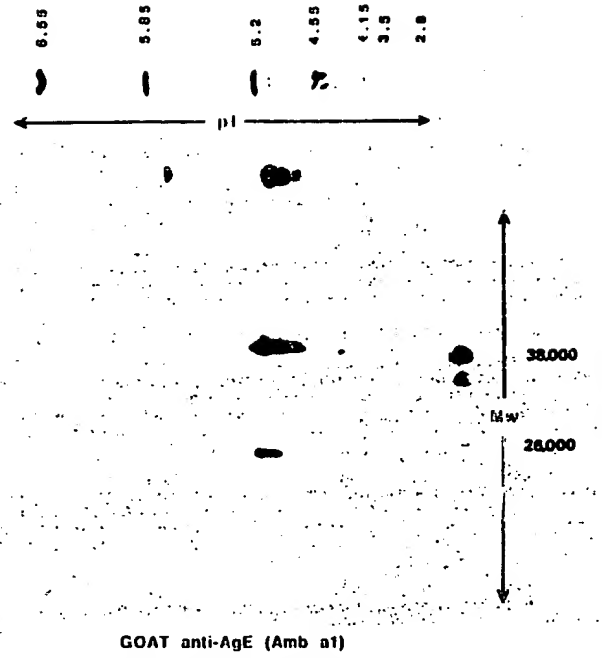


FIG. 3. **Two-dimensional gel electrophoresis and Western blotting of pollen protein.** Crude, soluble pollen proteins were subjected to isoelectric focusing (*left* to *right*) followed by 10% SDS-PAGE electrophoresis (*top* to *bottom*). The proteins were blotted onto nitrocellulose and probed with goat anti-*Amb a I* antisera. The 28,000 dalton band presumably represents the *a* chain of *Amb a I*.

FIG. 4. **RNA blot analysis of total RNA from various tissues.** 20 μg of total RNA was electrophoresed, blotted onto nitrocellulose, and hybridized to radiolabeled *Amb a I.2*. *Lane 1*, short ragweed pollen; *lane 2*, meadow fescue pollen.

FIG. 5. **DNA blot analysis of short ragweed genomic DNA.** Genomic DN. was digested with *Bam*HI (*lane 1*), *Eco*RI (*lane 2*), *Hin*dIII (*lane 3*), and *Nco*I (*lane 4*) and run on a 0.7% agarose gel. The DNA was transferred to nitrocellulose and hybridized to radiolabeled *Amb a I.2*.

the observed heterogeneity of *Amb a I* is due to allelic variation, multiple genes in a single plant, or perhaps both is presently under investigation.

DISCUSSION

The use of molecular biology techniques to study directly the structure-function relationships of allergens of medical importance has only recently begun to be exploited. Thus far three non-plant allergens (white-faced hornet venom antigen 5, bee venom phospholipase A2, and the house dust mite allergen *Der p I*) have been cloned and used for immunological studies (39–41). In this report, the major allergen of short ragweed pollen has been cloned, and its complete nucleic acid and deduced amino acid sequence are described. This allergen (*Amb a I*) is shown to be a family of proteins, closely related to each other but unique from other families of proteins.

Proteins in the pollen coat can originate from two different sources. They may be expressed in the pollen cytoplasm itself, or they can be synthesized in the tapetal nursing cells surrounding the pollen and then deposited on the pollen surface (42). cDNA libraries, therefore, were made from poly(A^+) RNA both from maturing flowers and from commercially available defatted ragweed pollen. The pollen cDNA library was made in λgt11 and was screened with a panel of monoclonal antibodies raised specifically against denatured *Amb a I*. From the information gained by cloning and sequencing *Amb a I* from this library, it was possible to probe a flower cDNA library made in λgt10. In addition, once sequence information was derived, it became possible to design oligonucleotide primers to amplify cDNA coding specifically for *Amb a I* from ragweed flowers by means of the PCR reaction.

Initially, a pool of seven monoclonal antibodies raised against denatured *Amb a I* (recognizing both α and β chains) was used to screen the pollen cDNA library. Three clones were isolated and fully characterized. One of these, *Amb a I.2*, contained an almost complete coding sequence for *Amb a I*, another named *Amb a I.3* contained approximately two-thirds of the coding sequence, and the third, *Amb a I.1*, contained 320 base pairs and coded for only 50 COOH-terminal amino acids of *Amb a I*. Further screening of the libraries (and PCR amplification of cDNA in the case of *Amb a I.1*) has produced full-length sequences of three groups of highly related structures representing *Amb a I*. A total of 14 clones were completely sequenced. On average, clones within each group differed at less than 1% of the nucleotides coding for the structural protein while the difference between groups was of the order of 10–15% at the nucleotide level. When full-length representative clones from each group are expressed in *Escherichia coli* and the products blotted onto nitrocellulose membranes for probing with monoclonal antibodies to denatured *Amb a I*, each gives a unique pattern of reactivity with individual antibodies (data not shown). This suggests that individual antibodies are able to discriminate between clones and demonstrates that all three groups of *Amb a I* proteins were in the chromatographically prepared *Amb a I* used to originally immunize the mice. These three groups of *Amb a I* clones, therefore, are expressed and are not transcripts of nonfunctional genes.

Multiple isoelectric forms and conservative amino acid substitutions are a common feature of pollen allergens that have been well characterized such as those from the grasses and from short ragweed (4, 43). It was shown earlier (4, 6) that *Amb a I* has four electrophoretic and/or structural forms called A, B, C, and D. These forms are indistinguishable by amino acid compositional analysis and comparison of their antigenic and allergenic properties. In this report, two-dimensional Western analysis of pollen extracts shows three major isoelectric forms of *Amb a I* and a number of minor forms that differ both in size and charge. This is consistent with the description of three groups of *Amb a I* clones and the appreciation that individual members of a group may differ by as much as 1% from the prototype within a group. Current efforts are directed at assigning specific groups to specific isoelectric forms of *Amb a I*. It is not clear at this time that the A, B, C, and D forms of *Amb a I* have any direct relationship to *Amb a I.1*, *Amb a I.2*, or *Amb a I.3* as described in this report.

Since the pollen used to create the cDNA library was collected from a wide geographic area, it is unlikely that other major *Amb a I* sequences will be found in short ragweed. Current experiments are under way to determine whether individual short ragweed plants collected from various geographical locations in North America express multiple (or all) members of the *Amb a I* family or whether individual plants show some restricted *Amb a I* production. Southern blot analysis of genomic DNA clearly shows multiple restriction fragments identified with a radiolabeled *Amb a I* probe, and it will be of interest to determine, in individual plants, whether allelic variation, multiple structural genes, or both contribute to this phenomenon.

Amino acid sequence analyses of peptides from chromatographically isolated *Amb a I* have been obtained. It is of great interest that the best match of that amino acid sequence is with the *Amb a I.1* group of *Amb a I* sequences. However, despite exhaustive screening of several independently derived cDNA libraries, only one short (320-base pair) clone belonging to this group was ever isolated, making this clone the most underrepresented member of the *Amb a I* family. A full-length clone of *Amb a I.1* was only obtained by sequence-specific amplification of pollen cDNA using primers specific for the 3′ end of the original 320-base pair clone and sequences shared between *Amb a I.2* and *Amb a I.3* at the 5′ end. Three possible explanations for this enigma are currently being considered. First, if it is the most actively transcribed and/or translated message, it could have a high turnover rate *in vivo* or be particularly prone to degradation during storage or processing of the pollen. Second, it is possible that its structure could somehow interfere uniquely with cDNA synthesis and cloning efficiency, and its paucity in the libraries merely represents a technical difficulty. Third, the *Amb a I.1* protein could in fact be a minor *Amb a I* variant but be selectively enriched for during the purification of *Amb a I* from pollen extract.

The amino acid composition of the cloned proteins corresponds well to experimental results reported previously (4). The presence of cysteine as the carboxyl-terminal deduced amino acid in all cDNA clones studied is in contrast to a report in which carboxypeptidase digestion of chromatographically purified *Amb a I* showed that leucine was the carboxyl-terminal amino acid (6). To settle the question of the carboxyl-terminal residue, the aspartic acid-proline peptide bond (position 360–361, Fig. 2) of chromatographically purified *Amb a I* was cleaved by 70% formic acid. The peptide sequence starting with proline was obtained in the digestion mixture whereas the nonspecific partially cleaved protein background sequence was suppressed by *o*-phthalaldehyde treatment. The 38-residue peptide sequence obtained corresponded completely with the deduced sequence of *Amb a I.1* up to and including the terminal cysteine.

Since the amino terminus of *Amb a I* is "blocked" (6) and not amenable to direct Edman degradation, it is important to be able to conclude that the clones reported here contain the full coding sequence for *Amb a I*. Two lines of evidence support that conclusion. The experimental evidence comes from a

previous study (44) in which primer extension was used to add nucleotides to the 3′ end of an oligonucleotide designed from a peptide sequence using fl wer mRNA as template. The 5′ end of the oligonucleotide corresponds to nucleotide 249 in clone *Amb a I.3* and produced a cDNA fragment about 250 nucleotides l ng, suggesting that very little 5′-untranslated sequence is missing from the full-length message. The second piece of evidence that these are full-length clones coding for *Amb a I* comes from a study of the area around the presumed translation initiation codon AUG (45, 46). From a study of 211 eucaryotic messages, a consensus sequence has been identified at the initiation site. The sequence contains a purine (preferably an A) at position −3, a G at position +4, and a predominance of C at positions −1, −2, −4 and −5. Clone *Amb a I.3* has A at −3, G at +4, and C at −2.

There is an obvious hydrophobic stretch of amino acids following the initiating methionine, and this area has been examined to predict the cleavage site between the hydrophobic leader and the secreted protein. The prediction is based upon data collected for 450 secreted eucaryotic proteins (47) with known cleavage sites. Positions −1 and −3 are most critical for signal peptide cleavage, and the alanine at position 26 in *Amb a I* best fulfills the criteria associated with the cleavage site. For instance, if alanine 26 is indeed residue +1, then the −1 and −3 positions are serine and valine, respectively. The frequency of those amino acids in those positions in the data base is two to three times the expected values if random amino acids occupied those positions. All amino acids, from −1 through −13, are consistent with the tabulations from the 450 known cleavage sites. Current efforts are under way to address directly the question of how and where the *Amb a I* proteins are blocked.

At the 3′ end, the untranslated region does not have the AATAAA polyadenylation signal observed in most animal sequences located 9–23 bases upstream from the poly(A) tail. However, there is a variant sequence, AATGAA, located 45 bases from the poly(A) addition site, which might serve as the adenylation signal; and another variation of this sequence, AAAAAT, is located 39 bases downstream from the TAA stop codon. A similar sequence, AATAAT is located 101 nucleotides upstream from the polyadenylation site in clone *Amb a I.2*. Poly(A) signal patterns are known to be much more complex in plants than animals (48) in their sequence, number, and locations. For example, a pollen-specific sequence from maize, Zmo13, reported by Hanson *et al.* (49), contains the consensus motif AATAAA 180 bases upstream from the poly(A) site and two variants, AATATA and AATTAT, located at 55 and 44 bases respectively. Another pollen-expressed gene, alcohol dehydrogenase, has the AATTAT sequence centered 44 bases upstream from its polyadenylation site.

The data presented in this paper firmly establish that *Amb a I* (antigen E) is a family of closely related proteins. Experiments are currently in progress to address the relative abundance of each family member in pollen, as mentioned above. Just as importantly, it is necessary to determine the immunogenicity and allergenicity of the *Amb a I* family members. It has been possible to express prototypic clones for each family member in procaryotic cells.[3] This material is being used to compare T cell proliferation and IgE binding from individual ragweed allergic patients. This information can potentially be used to devise an effective course of ragweed immunotherapy.

[3] J. Bond, R. Garman, K. Keating, T. Briner, T. Rafnar, D. Klapper, and B. Rogers, manuscript in preparation.

Acknowledgments—We wish to thank Drs. Joseph Olson, James Smith, and Robert Esch for providing reagents; Dr. David Marsh for providing goat anti-*Amb a I* IgG; Tom Wells, Anneliese Nault, Robert Schreifels, Andrew Brauer, and Joanne Pollock for technical assistance; and Joyce Bradshaw for preparing the manuscript.

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